43530

National Library of Canada / Bibliothèque nationale du Canada

CANADIAN THESES ON MICROFICHE / THÈSES CANADIENNES SUR MICROFICHE

NAME OF AUTHOR/*NOM DE L'AUTEUR* RONALD REHEL

TITLE OF THESIS/*TITRE DE LA THÈSE* EXERCISE INTENSITY AND DURATION ON PROTEINURIA

UNIVERSITY/*UNIVERSITÉ* UNIVERSITY OF ALBERTA

DEGREE FOR WHICH THESIS WAS PRESENTED/ *GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* M Sc

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE GRADE* 1979

NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* DR W. COTTLE

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

DATED/*DATÉ* May 11/79 SIGNED/*SIGNÉ* Ronald Rehel

PERMANENT ADDRESS/*RÉSIDENCE FIXÉ* 10697 BRUXELLES

MONTREAL

H1H 4R5

NL-91 (3-74)

National Library of Canada

Cataloguing Branch
Canadian Theses Division

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada

Direction du catalogage
Division des thèses canadiennes

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

THE UNIVERSITY OF ALBERTA

EXERCISE INTENSITY AND DURATION ON PROTEINURIA

by

(C) RONALD J. REHEL

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

AND RESEARCH IN PARTIAL FUFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF PHYSICAL EDUCATION

EDMONTON, ALBERTA

FALL, 1979

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled EXERCISE INTENSITY AND DURATION ON PROTEINURIA

submitted by RONALD J. REHEL

in partial fulfilment of the requirements for the degree of Master of Science in Physical Education.

May 4, 1979

Supervisor

Date W H Cottle

Dedicated to

my loving wife

ABSTRACT

Urine samples from ten trained cyclists were collected 20 to 30 minutes following exercise for the purpose of studying proteinuria with intensity and duration of exercise. All subjects completed four exercise conditions: A - 50% $\dot{V}O_2$ for 30 minutes, B - 50% $\dot{V}O_2$ for 60 minutes, C - 85% $\dot{V}O_2$ for 17.5 minutes, and D - 85% $\dot{V}O_2$ for 35 minutes. No significant differences ($p > .05$) in protein excretion rates were found between A and B, and between C and D, while significant differences ($p < .01$) were found between all other treatments. The results indicate the importance of exercise intensity on the protein excretion rate while exercise duration was of little value. The low intensity session resulted in excretion rates which approximated normal resting values.

ACKNOWLEDGEMENT

Sincere expressions of gratitude is extended to my adviser, Dr. W. Cottle, whose phone number is now indelibly imprinted in my memory. To the other members of my committee, Dr. A. Quinney, thank you for always having an open door, and Dr. K. Hutchison, thank you for coming to my rescue.

Thank you to Ted Wall, Andre Dorion, and Nancy Woods for their statistical consultation time.

I am left indebted to all the members of my family, my wife, parents, and brothers, whose expense of love, and support will leave me a millionaire forever.

To Bob Townsend of High Country Sport thank you for the bicycle equipment loan. To my 11 marvelous cyclists, whose ability to perform the vitals of life saved my thesis from going down the drain.

To my typist, Mrs. Cheryl Luchkow, I am forever in gratitude of your spelling ability and conscientiousness. Thank you!

Thank you Shirley Hilger for making lab work fun.

And finally, I acknowledge my many good friends, and particularly my buddy and colleague, Don Mattern, whose knowledge of proteinuria extends well beyond the laboratory.

TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
Statement of the Problem	5
Secondary Problem	5
Limitations	6
Experimental Error	6
Delimitations	6
Definition of Terms	7
REVIEW OF LITERATURE	9
Normal Urine	9
Urine Changes with Exercise	11
Exercise Proteinuria	13
Mechanisms and Related Factors Behind Exercise Proteinuria	21
Summary Statement	26
METHODOLOGY	27
Subjects	27
Apparatus	28
Procedures	28
Orientation Session	28
Test Session	29
Exercise Session	29
Urine Analysis	30
Dialysis Samples	31
Statistical Analysis	33

PAGE

RESULTS 38

DISCUSSION 46

SUMMARY AND CONCLUSIONS 51

REFERENCES 53

APPENDICES 60

APPENDIX A-I Physical Characteristics of Subjects 62

APPENDIX A-II Functional Characteristics of Subjects 63

APPENDIX B Health Questionnaire Form 65

APPENDIX C-I Scheffe Comparison of Mean Differences for Protein Excretion Rates During Four Exercise Sessions 68

APPENDIX C-II Protein Excretion Rates Following Exercise 69

APPENDIX C-III Summary of One-Way ANOVA on Protein Excretion Rates During Exercise Sessions 70

APPENDIX D-I Tests for Recovery of Added Protein Over the Course of Analysis 72

APPENDIX D-II Test for Recovery of Added Protein in Dialysis Analysis 73

APPENDIX E-I Raw Data - Exercise Session A 75

APPENDIX E-II Raw Data - Exercise Session B 76

APPENDIX E-III Raw Data - Exercise Session C 77

APPENDIX E-IV Raw Data - Exercise Session D 78

APPENDIX F Sample Data from Test Session 80

APPENDIX G Total Work Accumulated for Exercise Sessions 82

APPENDIX H-I Physical and Functional Characteristics for Case Study 84

APPENDIX H-II Raw Data for Case Study 84

PAGE

APPENDIX I Details of Chemical Constituents in Protein Analysis 86

LIST OF FIGURES

Figure		Page
1.	Protein Excretion Rate Following Four Exercise Sessions	40
2.	Relationship Between Oxygen Uptake and Power on Progressive-load Maximal Test	43
3.	Example of Protein Standard Showing Relationship Between Optical Density and Protein Quantity	45

LIST OF PLATES

Plate		Page
1.	The Modified Monark Bicycle Ergometer	35
2.	Cyclist in Riding Position During Gas Collection	35
3.	The Low Pressure Dialysis Assembly Used in Sample Concentration	37

INTRODUCTION

Approximately 180 litres of blood are subjected to filtration daily, yet only trace quantities of protein can be found in normal urine (Schultze and Heremans, 1966). Value of abnormal protein excretion (greater than 90 mg/24 hours, or more than .06 mg/minute) are usually viewed as indicating renal malfunction and are often linked with pathological consequence (Free and Free, 1975). However, exercise induces a transient proteinuria and is frequently accompanied by the appearance of red blood cells and increased numbers of cast formations. Gardner (1956) has described this condition as "athletic glomerulonephritis", and various other authors have also concluded the existence of exercise-pathology similarities. The modern expression "joggers' kidney" may well describe this condition, which because of increased numbers of jogging participants, is likely to affect a greater percentage of the population than heretofore. With the many reported benefits of exercise, it appears contradictory that exercise may be detrimental because of adverse effects on the kidney. There is a need for further information on the etiology of exercise proteinuria especially under chronic stress as is seen with the vigorous training undergone by athletes. It is nevertheless necessary to clarify the acute effects of exercise on the kidney. Fundamental to this is the need to establish the relationship between the amount of exercise, i.e. intensity versus duration, and the proteinuria it causes.

That proteinuria may occur with exercise is well established; however, whether it is the intensity or the duration of exercise which more greatly influences protein excretion, remains uncertain. A few studies,

such as Castenfors and Piscator (1967), Delforge et al. (1969), and Todorović et al. (1972), have studied two or more exercise intensities which were established through absolute heart rates or absolute power loads. Such a procedure allows for the possibility of large within-subject variation, which may in turn account for much of the wide dispersion of protein excretion results. Their studies suggest the importance of the intensity factor on exercise proteinuria. The relationship between duration of exercise and proteinuria appears not to have been investigated specifically. Studies on activities of short duration (Poortmans and Vancalck, 1978), moderate duration (Poortmans and Van Kerchove, 1962), and long duration (Poortmans and Jeanloz, 1968), reveal no apparent trend. The majority of studies, however, have focused attention on investigating renal function and possible mechanisms. The researchers, such as Javitt (1952) and Poortmans and Jeanloz (1968), have often used sporting activities or absolute work rates which offers little insight into the present problem. It seems that many conclusions on this topic have been based on speculation rather than observed results.

It is unclear whether the renal system can be trained. Taylor (1960) used the same repeated, absolute power load over the courses of several days and recorded a similar reduction in both heart rate and protein excretion rate on each succeeding day. Such evidence is supportive of the relationship between proteinuria and intensity rather than a renal adaptation to exercise.

Since urine flow with exercise can shift from an antidiuresis during exercise to a diuresis during recovery, two experimental precautions should be observed. Firstly, in preference to the frequent use of

protein concentration to quantify proteinuria, protein excretion rate is more indicative (Kachadorian et al., 1970; Poortmans, 1964). Secondly, Poortmans and Van Kerchove (1962) suggested the presence of a "washing-out phenomenon" which apparently occurs as a result of increased urine flow during the post-exercise period, and results in peak protein excretion rates at this time. The summarized findings of Poortmans and Van Kerchove (1962), Prejac et al. (1968), and Taylor (1960) suggest this peak protein excretion rate appears between 20 to 30 minutes following exercise.

The mechanism behind exercise proteinuria is controversial. It is known that plasma proteins constitute the greatest percentage of the total urinary proteins excreted both at rest and exercise, and their presence in the urine infers their passage through the glomerular filter as well as the passage through the tubules without tubular reabsorption. Various authors (Castenfors et al., 1967; Poortmans, 1969) have suggested an increase glomerular permeability to be the chief cause of exercise proteinuria. Such a cause would suggest that the tubular reabsorption mechanism becomes saturated; however, evidence found by Poortmans and Vancalck (1978) of a preferential or selective mechanism increases the importance of the tubular mechanism in determining the quantity of proteinuria found with exercise.

It has also been suggested that hemodynamic changes may explain exercise proteinuria. Boyarsky (1957) stated that the reduced renal plasma flow with exercise may leave the kidney ischemic which in turn may cause renal alterations. Exercise also causes an increased filtration pressure as is evidenced by increased filtration fraction (King and Baldwin, 1956). This, coupled with an increased plasma protein

concentration (Rocker et al., 1973), may then cause the glomerulus to be overloaded.

Various chemical agents are also suspected of being instrumental in producing exercise proteinuria. Notable among the suggestions is the renin-angiotensin II system (Božović et al., 1967). Their involvement may be explained by both the observed increase in plasma levels of these substances with exercise and the production of proteinuria as a result of infusion. Similarly, both catecholamines (Cantone and Cerretelli, 1960) and the kallikrein-kinin system (Hori et al., 1969) have also been included as possible responsible agents in exercise proteinuria. Finally, the change in blood pH with exercise, resulting from increased metabolic acid products, is believed to be a cause of exercise proteinuria (Javitt, 1952). This is accomplished through a possible increase in glomerular permeability.

The establishing of a relative parameter so as to monitor the stress encountered by body systems has been a problem in exercise physiology. Various studies have incorporated the absolute measurement of heart rate, absolute power loading, while others have adopted total work performed so as to quantify an exercise session. Although it may be preferred to consider one's anaerobic threshold in establishing a relative exercise measure, as suggested by Katch et al. (1978), methodological problems prevent the use of this procedure as a reliable practice. It is thus proposed that the subjects follow the various exercise sessions at relative percentages of their maximal oxygen uptake ($\dot{M}VO_2$). In the present study, two intensities, 50% and 85% $\dot{M}VO_2$ have been chosen to sample both extremes of the continuous exercise spectrum. Absolute exercise times have been chosen so as to compare the effects of duration

on the protein excretion rate.

Whether or not proteinuria with exercise reflects a potential danger to kidney function, as was suggested in many past studies, has not been substantiated. With the awareness of this possibility and the upsurgence in popularity for exercise programs; the indication is for further investigation. Proteinuria may in future prove to be a helpful tool for assessing and controlling levels of stress with exercise.

Statement of the Problem

The purpose of this study was to investigate the effects that various quantities of exercise have on the protein excretion rate in a group of trained cyclists. The study attempted to answer the following questions:

1. Is exercise proteinuria dependent on intensity or duration?
2. If intensity is the determining factor, then how does exercise proteinuria react quantitatively with a highly aerobic intensity level, and with another intensity, which is sufficiently more reliant on anaerobic processes?
3. If duration of a given bout of exercise is the determining factor, will it follow that prolonged exercise results in the greatest protein excretion?

Secondary Problem

A cyclist, who was found to have lost one kidney as a result of an

accident five years prior to the study, was also studied on two of the four exercise treatments. Although atypical, his response to exercise is of interest, and will be discussed in the appropriate section of this paper.

Limitations

The following limitations may have had an effect on this study:

- there was a limited number of subjects available.
- a homeogeneous group was sought, however a wide age range existed (19 to 32 years).
- it was left to the cooperation of the subject to uphold the outlined precautionary procedures in respect to diet, fluid intake, and exercise restrictions.

Experimental Error

A greater chance of experimental error existed for the ten samples which demanded dialysis treatment prior to analysis. It was assumed that the subjects were able to empty their bladder completely. Inability to do so would also constitute an experimental error.

Delimitations

This study dealt with highly trained athletes and whether or not the findings are applied to the general population awaits further evaluation.

Definition of Terms

Proteinuria: the presence of protein in the urine. The term is commonly used to express greater than normal levels of protein in the urine.

Normal proteinuria: the presence of normal levels of protein in the urine.

Proteinuria during exercise: the presence of increased protein in the urine immediately following exercise.

Post-exercise proteinuria: the presence of increased protein in the urine in the recovery period following exercise.

Exercise proteinuria: a term used to describe either proteinuria during exercise or post-exercise proteinuria.

Maximal oxygen uptake ($\dot{M}VO_2$): a term referring to the maximal volume of oxygen which can be consumed per minute (l/min or ml kg^{-1} min^{-1}).

Exercise session: describes one of the four exercise treatments used in this study. These include:

A - 50% $\dot{M}VO_2$ for 30 minutes

B - 50% $\dot{M}VO_2$ for 60 minutes

C - 85% $\dot{M}VO_2$ for 17.5 minutes

D - 85% $\dot{M}VO_2$ for 35 minutes

Test session: a session held several days prior to the exercise sessions, in which $\dot{M}VO_2$ was determined.

Anaerobic threshold: the stage during increasing levels of exercise at which functional anaerobic processes become evident, as seen with lactic acid increases.

Clearance (C): the theoretical volume of plasma which contained the mass of a substance that the kidney excreted per unit time.

Renal plasma flow (RPF): the rate of plasma flowing through both kidneys, which is commonly measured by the clearance of para-aminohippurate (C_{PAH}).

Glomerular filtration rate (GFR): the rate at which a glomerular filtrate is formed in all the nephrons of both kidneys, and is commonly measured by the clearance of inulin.

Filtration fraction (FF): refers to the fraction of the renal plasma flow which becomes glomerular filtrate (GFR/RPF).

REVIEW OF LITERATURE

For approximately a hundred years, science has been aware of the peculiar effect that exercise has on the renal system. The many works over the years have disclosed much information which has led towards a better comprehension of this complex area. Based on the nature and direction of these findings, the following review will be treated under the subsequent headings: normal urine, urine changes with exercise, exercise proteinuria, and mechanisms and related factors behind exercise proteinuria.

Normal Urine

Healthy adults can be expected to show minute levels of proteinuria without it being indicative of renal malfunction (Anderson, 1975). Various works (Boyce et al., 1954; MacGarry et al., 1955; Nebdal and Seliger, 1958; Poortmans, 1964; Rigas and Heller, 1951; Tidström, 1963) suggest a daily protein excretion rate ranging from 9 to 90 mg per 24 hours.

There is a variety of types of proteins which are found in normal urine. Grant (1959) arranged the urinary proteins into groups according to their origin. Four classifications evolved:

(1) a group of proteins corresponding to plasma proteins.

(2) a group of about six proteins which arise from the kidneys, ureters, and bladder.

(3) trace components from the male genital tract, especially the prostate.

(4) trace components common to both sexes, possibly arising from the urethra.

The plasma proteins, which constitute the greatest percentage of total urinary proteins both at rest and with exercise (Poortmans and Jeanloz, 1968), are said to be capable of passing through the glomerular filter, and are then possibly reabsorbed by the renal tubular epithelium (Schultze and Heremans, 1966). As stated by Poortmans (1972), the main feature of the urinary plasma proteins is that the only components present in normal urine in appreciable quantities, have molecular weights between 45,000 and 160,000. This feature may inevitably reflect the character of the glomerulus filter, which behaves physiologically as a semi-permeable membrane with a large number of pores per unit area, as seen with the findings of Rodewald and Karnovsky (1974), along with a possible electrostatic field composed primarily of sialic acid (Mohos and Skoza, 1970), and the added capability of repelling charged particles, as indicated by Chang et al. (1975). Proteins which escape the reabsorption process of the tubular epithelium are subsequently found in the urine.

A major protein material of postglomerular origin is the so-called Tamm-Horsfall mucoprotein. Also termed uromucoid, this mucoprotein has the tendency to be precipitated from solution by increasing salt concentration, and has been shown by Tamm and Horsfall (1950, 1952) to be effectively percipitated by .58 M NaCl. Solution acidity also decreases its solubility, as does albumin (McQueen, 1962). McQueen (1962) has shown in vitro that 1% albumin can precipitate 17% of the Tamm-Horsfall mucuprotein in normal urine, while other proteins, such as hemoglobin and γ- globulin, do not duplicate albumin's effectiveness as a

precipitator. In addition to this, McQueen (1962) showed this renal mucoprotein to be a major constituent of urinary casts. It is believed that casts are formed in the renal tubules.

Urine Changes with Exercise

The term albuminuria was once used by researchers in the belief that exercise resulted in high quantities of albumin excretion (Barach, 1910, 1920; Collier, 1907). Later studies (Cantone and Cerretelli, 1960; Nebdal and Seliger, 1958; Poortmans, 1964) have demonstrated, through the use of paper electrophoresis, that albumin-globulin ratios increased, and most recent research (Poortmans, 1964, 1975; Poortmans and Jeanloz, 1968) has indicated increases of several other urinary components consequent to exercise. While the albumin fraction remains the largest protein component of exercise proteinuria, high renal clearances of various other proteins have made the adoption of the "proteinuria" term the more suitable expression.

The presence of proteinuria, red blood cells, and casts are often used as indicators of renal disease, as discussed by Free and Free (1975). However, various works (Barach, 1920; Boone et al., 1955; Kachadorian et al., 1970) noted the increased incidence of red blood cells, hyaline and/or granular casts, as well as proteinuria following physical exertion. Moreover, Gardner (1956) found 50% of the 47 football players he studied also produced urine with elevated protein, red blood cells and casts, and noted the similarity of these samples to that of patients with glomerular nephritis. He thus coined the expression "athletic glomerular nephritis".

Other studies have also supported the pathological association. Kleiman (1960) reported the high incidence of proteinuria, casts and hematuria in some 1100 athletes following exercise, and on urological analysis of a smaller sample, described a condition "athletic kidney". This condition was characterized as having a distinctive renal lesion, a parenchymal defect. The author believed that intra-abdominal pressure may increase the circulating load on the nephron which could ultimately lead to morphological changes. A second proposal was that hydrostatic pressure increase, particularly as a result of a blow, could cause a rupture of the interlobar, arcuate, or intralobar veins and thus produce hematuria. Earlier, Alyea and Parish (1958) found the incidence of red blood cells to be similar for both the less traumatic exercise of distance swimmers as well as the heavily traumatic exercise of lacrosse players. The authors concluded that severe exercise produces a condition similar to that produced by mechanical trauma, while previously it was believed that trauma rather than exercise was the causative factor.

The presence of casts seen with exercise does differ from the disease condition. In contrast to the findings associated with acute renal failure, Patel (1964) found peak excretion of casts with exercise occurred during the period of oliguria and not subsequently during polyuria. The author believed that exercise allows for continuous flushing, while in acute renal failure, blocked tubules may delay urine passage. McKenzie et al. (1964) observed that increased periods of proteinuria in nephrotic patients were always associated with increased mucoprotein excretion. It may be that increased cast formation with exercise is not an indication of a pathological condition, but rather a result of the presence of increased precipitating agents, albumin,

decreased pH, and increased crystalloid concentration, which is accentuated through antidiuresis.

Exercise Proteinuria

Since the first known report on exercise proteinuria, that on marching soldiers by Leube in 1878 as cited in Castenfors (1967), most research has been directed towards understanding of the possible renal mechanisms. Few attempts have been made towards quantifying the exercise performed in relation to the amount of proteinuria produced. Also, the effectiveness of using urinary protein concentration as a quantification expression for proteinuria is limited, in view of the antidiuretic effect associated with exercise. The use of protein excretion rate is advocated by Kachadorian et al. (1970) and Poortmans (1964).

Protein excretion rates are found to be higher in the post exercise period rather than immediately following the exercise. Poortmans and Van Kerchove (1962) showed that urinary flow dropped from an average 1 ml/minute at rest, prior to an 8 km race, to .48 ml/minute immediately following. The protein excretion rate also increased from a mean of .029 mg/minute to .99 mg/minute for these respective times. In addition, the 30 minute post exercise sample showed an increased urinary flow to 2.2 ml/minute, along with a coincidental elevation in protein excretion rate to 3.96 mg/minute. These authors hypothesized that the increased urinary flow in the recovery period may overwhelm the tubular reabsorptive capacity, resulting in increased protein excretion. This occurrence was termed the "washing-out" phenomenon.

Prejac et al. (1968) further substantiated the existence of an increased excretion rate in the recovery period. At an absolute power load of 200 watts for 30 minutes, 16 subjects showed increased protein excretion, from an average .13 mg/minute pre-exercise value to .25 mg/minute at three minutes post-exercise, which was taken to reflect the exercise period. Peak protein excretion of urinary protein occurred with the first 20 minute collection period, a value of greater than .35 mg/minute. Pre-exercise levels were resumed 60 to 120 minutes following exercise. Earlier, Taylor (1960) had had similar findings, with a peak excretion of approximately .26 mg/minute with the first 20 minute sample, as compared to a value of about .1 mg/minute immediately following exercise. Normal protein excretion rate occurred 60 minutes following the exercise which consisted of 15 minutes on the treadmill at five miles per hour, on a one-in-seven gradient.

There appears to be evidence that protein excretion rates may remain elevated for a considerable period following exercise. Coye and Rosandich (1960) collected urine samples for 24 hours following a football practice. It is unclear whether each urine micturition was analyzed separately or combined to make a collective 24 hour sample; thus the mean protein excretion rate of 14.05 mg/hour may not reflect a high protein excretion for the full 24 hour period. Nonetheless, the authors concluded that two phases of exercise proteinuria exist; an initial phase of heavy protein excretion and a second longer phase of less excretion. They postulated that this second phase is the result of either delayed repair of a glomerular defect or impaired tubular reabsorption of the filtered protein following renal ischemia. Various other works (Castenfors and Piscator, 1967; Poortmans and Vancalck, 1978; Prejac et al., 1968;

Taylor, 1960) reported that total protein excretion values normalize within two hours following exercise.

Many researchers have investigated the protein components excreted in urine, so as to gain some insight into glomerular and tubular function, as for example Boesken et al. (1973). The occurrence of large molecular weight proteins in exercise urine, which are uncommon to normal urine, infers a possible increase in glomerular permeability. In normal urine, Berggård and Bearn (1962) found only haptoglobulin type 1-1, which is a small fraction of haptoglobulin with a molecular weight of 100,000. However, haptoglobulin type 2-1, of molecular weight 220,000, was observed in urine samples following a 9 km race (Poortmans and Segers, 1964). Furthermore, Poortmans and Jeanloz (1969) reported an increased incidence of γD-globulin, as well as increased γG- and γA-globulins following a 26.2 mile marathon race. The authors state that an increased glomerular permeability may explain the increased excretion of these filtered immunoglobulins. It would be noted that A-globulin has also been thought to be of renal secretory origin, thus partly accounting for its high renal clearance at rest (T[illegible]asi et al., 1965). It seems infinitely difficult to establish whether hemodynamic changes or increased glomerular permeability per se, offers the greatest contribution to the passage of large molecular weight proteins which are present in normal urine and increased in exercise samples.

Study of the tubular reabsorption mechanism is often accomplished through the investigation of small molecular weight proteins. While Schultze and Heremans (1966) presented a model explaining tubular reabsorption of proteins as a non-selective process, with a constant 95% reabsorption for the various proteins, Peterson et al. (1969), from

several lines of evidence, proposed selective tubular reabsorption indeed existed for albumin and β_2-microglobulin in normal subjects at rest. Also, while it was thought that exercise proteinuria could be explained chiefly in terms of changes in glomerular function with a saturation of the tubular reabsorption capacity (Castenfors et al., 1967; Poortmans, 1969), various other findings support a preferential tubular reabsorption mechanism favouring certain proteins (Poortmans, 1972; Poortmans and Vancalck, 1978).

Support of a non-selective tubular reabsorption mechanism was shown by Castenfors et al. (1967), who found no marked change in the excretion rates of α-amino acid N and ribonuclease after an 85 km ski race. These small molecular weight nitrogenous compounds are expected to be freely filterable at the glomerular membrane, and therefore changes in the excretion would reflect changes in their tubular reabsorption. With the assumption that the filtered load remained constant, tubular reabsorption was unchanged for the above compounds. Castenfors and Piscator (1967) also saw basically no change in ribonuclease excretion with light and heavy load supine cycling. The authors agreed that exercise proteinuria was reliant on glomerular rather than tubular changes.

Investigations of various other proteins suggest a selective tubular reabsorption with exercise. Poortmans' (1975) review cites a study of cyclists who exercised at 2/3 $\dot{M}VO_2$ for 60 minutes. Values revealed no change in the urinary excretion rate of ribonuclease or β-glucuronidase from rest to 30 minutes post-exercise samples. However, amylase and lysozyme excretion rates were significantly higher for the 30 minute sample. Although the author did not report the clearance values, it is assumed that a selective tubular reabsorption exists. More conclusive

results are shown by Poortmans (1972) for nine males and eight females, following six one-minute, maximum stress-rest intervals on the bicycle. Lysozyme (molecular weight 15,000) and amylase (molecular weight 50,000) showed increased urinary excretion rates, although only lysozyme showed an increased clearance. Thus the increased lysozyme excretion cannot be explained by increased filtration; the author suspected that change in tubular reabsorption may have accounted for the change in clearances of these two proteins.

It seems that slightly larger molecular weight proteins may also have varied clearances following exercise. Following a marathon, the calculated renal clearance of α_1-acid glycoprotein with a molecular weight of 44,000, showed a renal clearance of approximately 21 1/minute, as compared to less than .5 1/minute at rest. This difference indicates a 42-fold increase. However, β_2-glycoprotein I (weight 40,000) recorded less than a two-fold increase, with the renal clearance being less than 2.0 1/minute after exercise compared to approximately 1.1 1/minute at rest. The authors explain these results by suggesting a preferential tubular reabsorption, and/or penetration of proteins into the nephron at post-glomerular sites.

Protein tubular reabsorption may be a selective process during peak exercise proteinuria, as well as into late recovery when protein excretion values may again be near normal. Poortmans and Vancalck (1978) followed 15 females through seven one-minute maximal exercise-rest intervals on the bicycle. Urine samples taken pre-exercise, as well as 15 and 45 minutes post-exercise, showed a mean peak protein excretion of 388 g/minute at 15 minutes post-exercise, and only 109 g/minute for the 45 minute post-exercise sample. Estimated renal excretion rates

and clearances for albumin and β_2-microglobulin also showed peak values with the 15 minute sample, and remained elevated for the 45 minute sample as well. These same authors further proposed that increased β_2-microglobulin-albumin ratios with exercise indicate tubular selectivity in reabsorbing the proteins, and that increased albumin-total protein ratios may suggest increased glomerular permeability. Thus, exercise proteinuria was concluded as glomerular-tubular in origin. It should be noted that if renal clearance ratios of these proteins had been cited, clarification of tubular function during and following exercise would be greatly facilitated.

While it may be generally believed that the quantity of proteinuria produced is somewhat dependent on the quantity of exercise performed, the exact relationship is nebulous. Fries and Jundell in a 1911 paper cited in Christensen and Högberg (1950), concluded that proteinuria is dependent on exercise intensity, and independent of duration, while in contrast, Delanne (1957) cited in Poortmans (1964), found proteinuria to be a function of both exercise intensity and duration. Lack of quantification of both parameters has obscured interpretation of results.

Exercise intensity seems to be related to the protein excretion rate. Castenfors and Piscator (1967) studied 11 subjects during 45 minutes of supine cycling on both light and heavy loads. Subjects were tested on supine work capacity, which is the power load necessary to obtain a heart rate of 170 beats per minute (W 170); a light load (range 30 to 58% W 170) and heavy power loading (range 63 to 89% W 170) were then established so as to elicit heart rates of 120 and 160 beats per minute respectively. The corresponding peak protein excretion rates were 250 g/minute and 350 g/minute. The authors did not report the

range and deviation of the proteinuria values; however, it is expected that great individual differences would have been present. Also, the close scheduling of the light and heavy exercise sessions (1 hour interval) confuses interpretation of results. In a similar study, Delforge et al. (1969) followed six subjects on the bicycle ergometer at various intensities, "120W, 140W, 160W, and 180W per minute [sic]" for periods of 15 minutes over consecutive days. These authors used a resting proteinuria value which was not quantitatively specified, to represent a 100% value, and exercise protein excretions were then recorded as percentages of this base value. Their findings showed 156, 185, 261, and 547 to be the percentages of resting values for the respective forementioned intensities, for samples taken immediately following exercise. It is unclear whether the measurement of resting proteinuria indicated an excretion rate or simply a protein concentration. Thirty minutes post-exercise values showed a reduced percentage of rest values for the final three test conditions, and in light of the previous studies which suggest a post-exercise diuresis, then this representation of proteinuria is believed to be based on protein concentration. Conclusive evidence, therefore, is not available with this data.

It may be that a certain exercise quantity is required before proteinuria is elicited. Studying 499 males from 10 to 69 years of age, Perlman et al. (1970) used treadmill walking with subsequent gradients elevations until exhaustion, or until achieving a predetermined target heart rate according to age. Proteinuria was measured by a protein indicator, Albustix. Covariant analysis, with age held constant, showed absolute oxygen consumption at 150 beats per minute, total time of exercise, systolic blood pressure and maximum heart rate reached, to be

all significantly correlated with the occurrence of post-exercise proteinuria. The authors speculated that heart rate, oxygen uptake, and time of exercise are factors which may increase glomerular permeability by affecting body temperature, acid-base balance, and hormone secretion.

Proteinuria may not necessarily depend on the amount of work performed. Todorović et al. (1972) pretested 11 males on physical work capacity, which is the power load required to elicit a heart rate of 170 beats per minute (PWC 170), and then established two exercise sessions of similar work total. Work I was, however, continuous for 15 minutes and so established as to raise the heart rate to 170 beats per minute in the last minute of exercise. Work II was also 15 minutes in duration but with varied intensity and no rest intervals. The total urinary protein excreted was 11.0 ± 6.7 mg for Work I, and 13.2 ± 7.6 mg for Work II, for the 30 minutes after exercise. No significance was seen, possibly due to the scattering of results, which may have reflected the groups' exercise at an absolute heart rate. Nonetheless, the authors concluded that proteinuria seemed related directly to intensity and duration of the work. This may, in fact, be an unjustified conclusion.

Training studies with an aim of demonstrating renal adaptation to exercise, have shown exercise proteinuria to be related to exercise intensity. Taylor (1960) had eight subjects repeat a 15 minute treadmill run at seven miles per hour with a gradient of one-in-seven, for each of five days. Each successive day showed a reduction in heart rate with an associated decrease in the protein excretion rate. Since the subjects trained each day at a relative decrease in exercise

intensity, it is not evident whether or not a renal training effect was actually achieved, while these findings do indicate a relation between exercise intensity and exercise proteinuria.

Various urinary protein excretion rates are also seen in athletic endeavours. After an 8 km race, 30 minutes post-exercise samples for the athletes showed marked elevations in urinary protein excretion rates, averaging almost 4 mg/minute (Poortmans and Van Kerchove, 1962), while the similar 30 minute post-exercise samples of Boston marathon athletes showed an average protein excretion rate of only .213 mg/minute (Poortmans and Jeanloz, 1968). McKay and Slater (1962) recorded a maximum protein excretion rate of 5.1 mg/minute in swimmers. Cyclists, while exercising at 2/3 MVO_2 for 60 minutes, showed an increase in all seven measured protein components 30 minutes following exercise. An approximately two-fold average increase was seen in the seven components.

Mechanisms and Related Factors Behind Exercise Proteinuria

The phenomenon of proteinuria with exercise has been linked to hemodynamic changes as well as involvement of chemical substances. Generally, the hemodynamic changes include reduction of renal plasma flow, decreased glomerular filtration rate, increase in filtration pressure, increased plasma protein concentration, and increased blood acidity. The involvement of chemical substances such as norepinephrine and epinephrine, renin and angiotensin II, kallikreins and kinins have been established partly on their elevated plasma levels or increased urinary excretion with exercise, also the evidence of increased proteinuria with infusion of these substances. All these factors are seemingly

related, and a single responsible factor, at this time, cannot be isolated for exercise proteinuria.

Various works support the finding of a decreased renal plasma flow with exercise (Castenfors and Piscator, 1967; Chapman et al., 1948; Grimby, 1965). Grimby (1965) found that the renal fraction was 17% of the cardiac output and this figure decreased to 2.5 to 5% with increasing power loads. Furthermore, Castenfors and Piscator (1967) found the decrease in renal plasma flow with exercise was thought to leave the kidney ischemic, as seen in disease states (Boyarsky, 1957). Increased proteinuria is a sign associated with both situations.

Regardless of a decreased renal plasma flow resulting from exercise, the oxygen supply still exceeds the metabolic demand, as suggested by Zaruba and Fixa (1964) through their observations made on dogs. The authors proposed the existence of a selective renal ischemia of exercise. Earlier, White and Rolf (1948), while noting the drop of the clearance of the para-aminohippuric acid (C_{PAH}) with exercise, also questioned whether this decrease actually reflected a uniform reduction of blood flow through the various vascular channels. Their suspicion was based on the "apparent" absence of proteinuria during the exercise session, which inferred a differential constriction of various renal vascular channels. However, existence of a possible selective renal ischemia is reported by Göthlin et al. (1975). Using varying doses of arginine vasopressin, infusions were performed on rabbits which caused the glomeruli to shut off to an increasing degree, beginning in the outer cortex. Earlier, Thorburn et al. (1963) while studying dogs under normal conditions, showed the renal cortical blood flow to be about 80% of the total renal blood flow. The outer medulla revieved 16% of

the flow, while the inner medulla had only 2% of the total. If the change in renal blood flow is non-uniform in exercise, then a selective ischemia may be possible.

Exercise proteinuria may be the result of catecholamine involvement. Gray and Beetham (1957) saw a marked increase in plasma norepinephrine, rather than epinephrine, following exercise; Kotchen et al. (1971) further found exercise intensity to be a factor in the level of norepinephrine produced. Epinephrine was increased only with severe exertion. These findings are in agreement with Howley (1976) who demonstrated similar results in urinary excretion levels. King and Baldwin (1956) showed reductions in renal plasma flow and glomerular filtration rate, but increases in filtration fraction following the infusion of L-norepinephrine as well as for epinephrine. However, the qualitative proteinuria test was positive following norepinephrine infusion in a greater percentage of the cases. The calculated renal vascular resistance consisting of total renal resistance, afferent resistance, net efferant resistance and venular resistance were all increased following infusions of both catecholamines. However, the norepinephrine infusions' greatest contribution to total renal resistance was a marked increase in efferent resistance, while epinephrine infusions greatly increased the venular resistance. The authors (King and Baldwin, 1956) claim that elevated intraglomerular pressure is evidenced by the increased filtration fraction and the increase in net efferent resistance. This suggests that there exists a hydrostatic pressure favouring diffusion of protein through the glomerular membrane. Reports of exercise studies (Castenfors and Piscator, 1967; Chapman et al., 1948; Kotchen et al., 1971; Zaruba and Fixa, 1964) have also indicated increases in filtration

fraction. Coupled with an increased plasma protein concentration as seen with exercise (Poortmans, 1971; Röcker et al., 1973; Saltin and Stenberg, 1964), this increase in filtration pressure may cause a protein loading situation on the glomerulus.

Evidence exists that proteinuria may be stimulated through the production of renin. Diverse works (Aurell and Vikgren, 1971; Božović et al., 1967; Kotchen et al., 1971) have noted increased plasma renin levels with exercise. Specifically, Kotchen et al. (1971) found similar responses for both plasma renin and norepinephrine levels with ten minutes exercise at 40%, 70%, and 100% $\dot{M}VO_2$ and significant increases were seen at 70% and 100% only. Thus the magnitude of renin response seems related to the exercise intensity. Also, through the infusion of renin (Sellers et al., 1952; Deodhar et al., 1964) or angiotensin II (Eisenbach et al., 1975; Hori et al., 1969), increased proteinuria was produced. Since renin facilitates the production of angiotensin II both are thought to produce the same proteinuria-inducing effect on their infusion. The action of these substances remains controversial. Some studies have supported the view that angiotensin II increases glomerular permeability, as seen by the use of marker molecules and the electron microscope observations by Sellers et al. (1952) and Deodhar et al. (1964), and further substantiated with the micropuncture studies by Eisenbach et al. (1975). Opposition to this view by Pessina et al. (1972) and by Bohrer et al. (1977) with their studies using synthetic tracer molecules, reveal that the increased proteinuria could be explained primarily by hemodynamic factors, and not by glomerular permeability. Clarification of this area may help to explain the mechanism behind exercise proteinuria.

Although not widely documented, data exists that the kallikrein-kinin system may also be responsible for exercise proteinuria. Murakami et al. (1968) reported a two-fold increase in urine kallikrein excretion as well as a similar increase in proteinuria with exercise. Urinary histamine excretion was likewise increased, and with a subsequent series of infusion studies using simomenine, a histamine liberator as a pre-medication, it was concluded that both kallikrein and histamine were responsible for kallikrein-induced proteinuria. Further study of these vasoactive peptides by Hori et al. (1969) suggested disimilarities between angiotensin II-induced proteinuria and the kallikrein-induced state. Premedication of aldosterone or spironolactone, a diuretic, produced no effect on kallikrein-induced proteinuria, while the opposite was true for antiotensin II-induced proteinuria. The authors suggest that both these systems may be responsible for exercise proteinuria.

Changes in acid-base balance may partly be responsible for exercise proteinuria. Barach (1920) found that although 85% of all urine samples from baseball players were acidic, the highest urine acidity did not determine the presence of albumin, blood cells, or casts. Javitt (1952) also found that protein excretion rate did not remain elevated throughout the maximal depression of urine pH; however, blood acidity was significantly correlated with protein excretion rate. Furthermore, prealkalinization of subjects with sodium bicarbonate did not produce a reduction in protein excretion rate. Similar findings were also made in studies on the male rat, where metabolic acidosis and alkalosis have been shown to alter the urinary protein excretion (Gardner, 1961). The early in vitro work by Chambers and Zweifach (1940) suggested that decreased pH may have resulted in an increased glomerular permeability through

possible alterations in intercellular cement. However, Cantone and Cerretelli (1960) reported that administration of ammonium chloride produced the desired decrease in blood pH, but resulted in little change in urinary protein concentration. Although Todorović et al. (1972) found that for continuous exercise (Work I) significant correlations exist between protein excretion during the 30 minute post-exercise period and blood pH, the same relationship was not present for exercise of variable intensity even though it was equal in work to that of Work I. Todorović et al. (1972) concluded that once the highest proteinuria level is attained, further decreases in blood pH bear no relation to the proteinuria level.

Summary Statement

As can be seen, the area of proteinuria resulting from exercise is not completely understood. From the research discussed, however, various points have evolved. The quantity of exercise proteinuria seems related to the intensity of the exercise, while little evidence exists linking the duration of exercise to the protein excretion. Also, the proteinuria produced may have evolved from any of a combination of changes, including an increased glomerular permeability, the presence of a saturated, decreased, or selective tubular reabsorption, and hemodynamic changes with resulting filtration pressure and plasma concentration increases. Various related factors may have produced these described changes. These include known vasoactive substances, catecholamines, renin-angiotensin II, and kinin-kallikrein, as well as influences produced by increased blood acidity. No conclusive statements may presently be made in this complex area, and further research is required.

METHODOLOGY

To evaluate the effects that various quantities of exercise have on the protein excretion rate, a group of cyclists were studied. Each subject was tested for maximal oxygen uptake ($\dot{M}VO_2$) during a test session, which served to establish the work rates for four exercise conditions (exercise sessions). Proteinuria was studied with the four following exercise sessions:

A - 50% $\dot{M}VO_2$ for 30 minutes

B - 50% $\dot{M}VO_2$ for 60 minutes

C - 85% $\dot{M}VO_2$ for 17.5 minutes

D - 85% $\dot{M}VO_2$ for 35 minutes

The exercise sessions were so designed that the total relative energy expenditure of a low intensity session would approximate that of a high intensity session. Specifically, session A was equated to C, and B with D, with the provision that subjects were capable of maintaining the same high work rate at an 85% $\dot{M}VO_2$ for the entire session.

Subjects

Ten cyclists from the Edmonton Road and Track Club acted as subjects. They ranged in age from 19 to 32 years ($\bar{x}$ = 25.5 $\pm$ 4.3 years), and in weight from 65.7 to 80.4 kg ($\bar{x}$ = 74.7 $\pm$ 4.9 kg). The relative $\dot{M}VO_2$ values were from 51.7 to 67.2 ml $kg^{-1}min^{-1}$ (61.8 $\pm$ 1.8 ml $kg^{-1}min^{-1}$). For physical and functional characteristics see Appendix A.

The subjects were well trained, cycling approximately 100 to 300 miles per week during peak training season, as well as engaging in

regular competition. The commencement of the study occurred at the termination of the competitive season. Although subjects maintained an exercise regime throughout the course of the study, they were instructed to refrain from undue activity on the day of the test or exercise session.

Apparatus

During both the test and exercise sessions, the subject's expired gas was routed to a Metabolic Measurement Cart (Beckman Instruments Inc., Illinois) for ongoing analysis. Calibration of this instrument was performed prior to each use.

During the test session, an electrocardiograph (ECG), Sanborn 500 Viso-Cardiette), was used to monitor the subject's heart response. During exercise sessions, heart rates were monitored directly by a Cardiometer (Cardionics, Stockholm, Sweden).

For this study, the Monarch ergometer was modified by using racing-style handlebars, racing seat, and pedals equipped with foot stirrups. This modified ergometer was found to be more comfortable as it more closely resembled the bicycle used customarily by the cyclist (see Plate 1).

A Pye Unicam SP-1800 Ultraviolet Spectrophotometer was used to measure optical densities during protein analysis of the urine samples.

Procedures

Orientation Session

In order to familiarize the subjects with the procedures of the study, an orientation session was held. Also at this time, subjects

completed a health questionnaire (see Appendix B) which served as a screening procedure, and were measured on various physical characteristics including arterial blood pressure, and body fat estimation by underwater weighing.

Test Session

Subjects were initially fitted with ECG electrodes, nose clip and mouthpiece, and were given a 10 minute warm-up period at a force of 1.5 kp pedalling at 70 revolutions per minute (rpm). The seat positioning was left to the subject's discretion.

The $\dot{M}\dot{V}O_2$ test was a graded test with no definite time period for each power load. Subjects were allowed to reach steady state during the submaximal loads. Increments of power load were then matched with the corresponding elevation in $\dot{V}O_2$, as for example, the elevation of 150 kpm per minute is said to elicit a 300 ml/minute increase in $\dot{V}O_2$, according to Issekutz et al. (1962). Towards the end of the test, the attainment of the expected increase in $\dot{V}O_2$ for the corresponding power increment served as the criterion for further power increases. Maximal oxygen uptake was considered achieved when a "levelling off" effect was observed, as described by Hickson et al. (1977).

Maximal power output (MPO) for a corresponding $\dot{M}\dot{V}O_2$ was calculated by the extrapolation of a line linking steady state $\dot{V}O_2$ values for submaximal power loads until intersection with the $\dot{M}\dot{V}O_2$ level. This MPO value then served to establish the initial force setting corresponding to 40%, 50%, and 85% $\dot{M}\dot{V}O_2$ for the exercise session.

Exercise Session

Within five days of the test session, each subject was assigned the four exercise sessions in a random order, and commenced the sessions

which were separated by two day intervals.

A warm-up period of five minutes at 40% MVO_2 was given to each subject just prior to the exercise session. Three minutes following the completion of the initial period, the subject emptied his bladder and this voiding served as an initial time marker.

Once urination was completed, the exercise session commenced and was continuous from the start to the termination of the test. The first of a series of $\dot{V}O_2$ measurements occurred during the early minutes of this session. Once a heart rate was linked with the required $\dot{V}O_2$ value predetermined for the particular session, the subject was disconnected from the mouthpiece, and the procedure was repeated periodically during the course of the exercise. When the exercise session was completed, the subject was allowed to cycle against no load until his heart rate had returned to 120 beats or less per minute. The subject then sat quietly until urination time 20 to 30 minutes later.

It was observed that heart rate could be maintained within $\pm$ 2 beats per minute while the oxygen uptake, taken as an average of 4 consecutive half-minute measurements, could be maintained within $\pm$ 75 ml/minute of the target value. Plate 2 illustrates the cycling position during the testing session.

Urine Analysis

Urine collections were made 20 to 30 minutes following exercise termination through a volitional and as complete as possible voiding. The collection volumes were immediately measured and sodium azide, as preservative, was then added to a final concentration of 1 mg/ml. Stored in clear pastic containers, these samples were then quickly frozen at -65°C, and then later transferred to a -35°C freezer for long-term storage.

For analysis, samples were first allowed to thaw at room temperature, a procedure which required approximately three hours. Uromucoid was removed through filtration using Whatman's No. 1 filtering paper, as indicated by Berggard (1962). Analysis was then carried out in triplicate. As described by Piscator (1962) to precipitate the protein, equal volumes of the filtered urine and Tsuchiya's reagent were mixed. After vortexing, the mixture was allowed to stand for 15 minutes then centrifuged at 1800 g for 15 minutes. The protein precipitate thus formed was then washed twice with 95% ethanol and recentrifuged following both washes. The ethanol was thoroughly decanted and the precipitate was redissolved in 4 ml of 3% sodium hydroxide (NaOH) and combined with .2 ml of Benedict's reagent, as described by Goa (1953) and Piscator (1962). Colour was allowed to develop for 15 minutes, then absorption was read at wavelength 330 λ, against a blank 4 ml NaOH and .2 ml Benedict's reagent).

For protein standards, human serum albumin was prepared with 4 ml of 3% NaOH and .2 ml of Benedict's reagent. A standard was run with each day's analysis. To evaluate the effectiveness of the precipitating procedure, known concentrations of human serum albumin in a buffer solution were prepared and subjected to the above described procedure, and compared to the protein standard.

Dialysis Samples

The protein concentration of 10 exercise samples were too low for effective precipitation. It was therefore necessary to concentrate these particular samples through dialysis. This was accomplished using a modification of the method of Everall and Wright (1958), which utilized low pressure filtration through dialysis tubing. The particular tubing

used, Spectrapor 3, had a slightly larger diameter, 11.5 mm, and lower molecular weight cutoff point, 3500, than the original ½ inch Visking tubing used by Everall and Wright (1958). The tubing was prewashed in water for two hours prior to use.

Apparatus used in low-pressure ultrafiltration is shown in Plate 3. An assembly using a 1 to 2 l aspirator flask along with a long-spouted funnel and rubber stopper was set up in a cold chamber at 5°C. Specifically, both the spout and one end of the dialysis tubing were passed through a drilled hole in the rubber stopper so that both items were held securely by the stopper. The tubing then was formed into a loop with the remaining end being between the stopper and the neck of the flask. It was found more satisfactory to use a lower negative pressure than the original 600 mm Hg. A bleed orifice was used to correct for pressure changes in the vacuum line and thus the exact negative pressure was not determined. Dialysis of a 100 ml sample to 5 ml was accomplished in a period of two to three days.

Urine samples were combined with a phosphate buffer pH 7.0, up to a volume of 100 ml. Such a procedure was adopted to reduce experimental error. The section of dialysis tubing which contained the undialyzed portion of the sample was removed and then placed into a conical centrifuge tube so that one end was sealed securely to the rim of the centrifuge tube while the other end remained freely in the bottom. This assembly was centrifuged so as to thoroughly remove the contents from the dialysis tubing. The volume was carefully measured by pipetting. The filtrate contained in the aspirator flask was also measured for volume using a graduated cylinder.

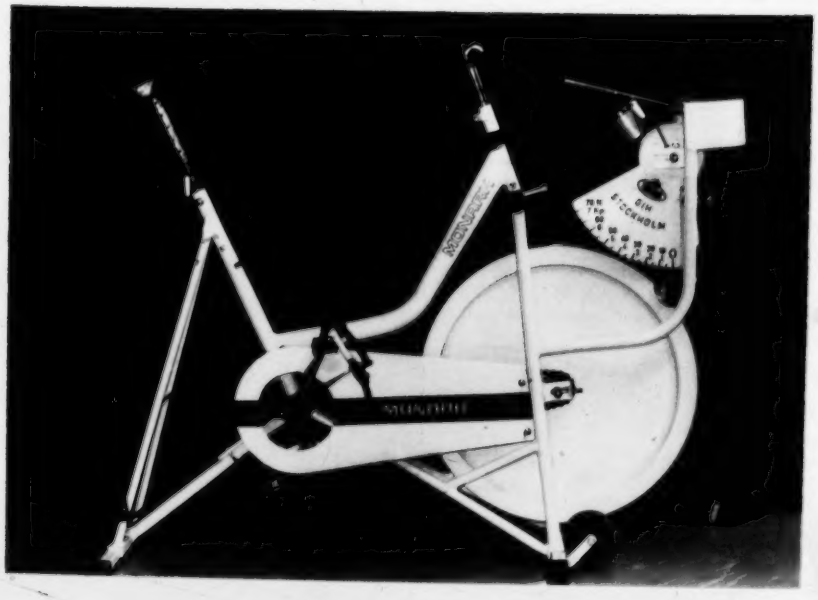
Analysis of the concentrated samples was performed as previously reported.

Statistical Analysis

The statistical analysis of the results involved a one way ANOVA with repeated measures. The statistical computations were made using a ANOV 14 (Ders program). Post hoc procedures involved a Scheffe for locating mean differences. Significant differences were accepted at the alpha level of .05 ($p < .05$ where p is the probability that no difference exists between the means).

Plate 1. The Modified Monark
Bicycle Ergometer

Plate 2. Cyclist in "Riding Position"
During Gas Collection



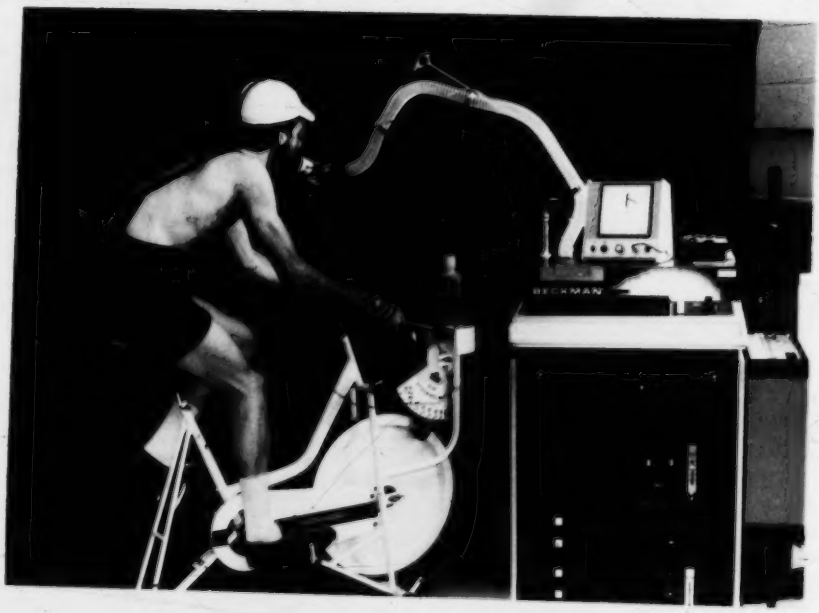
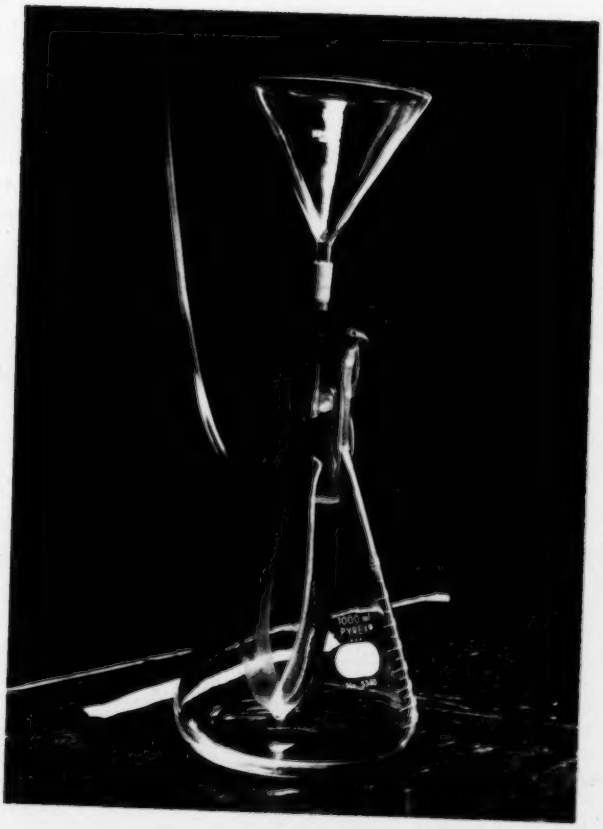

BECKMAN

Plate 3. The Low-Pressure Dialysis Assembly Used in Sample Concentration


1000 ml
PYREX

RESULTS

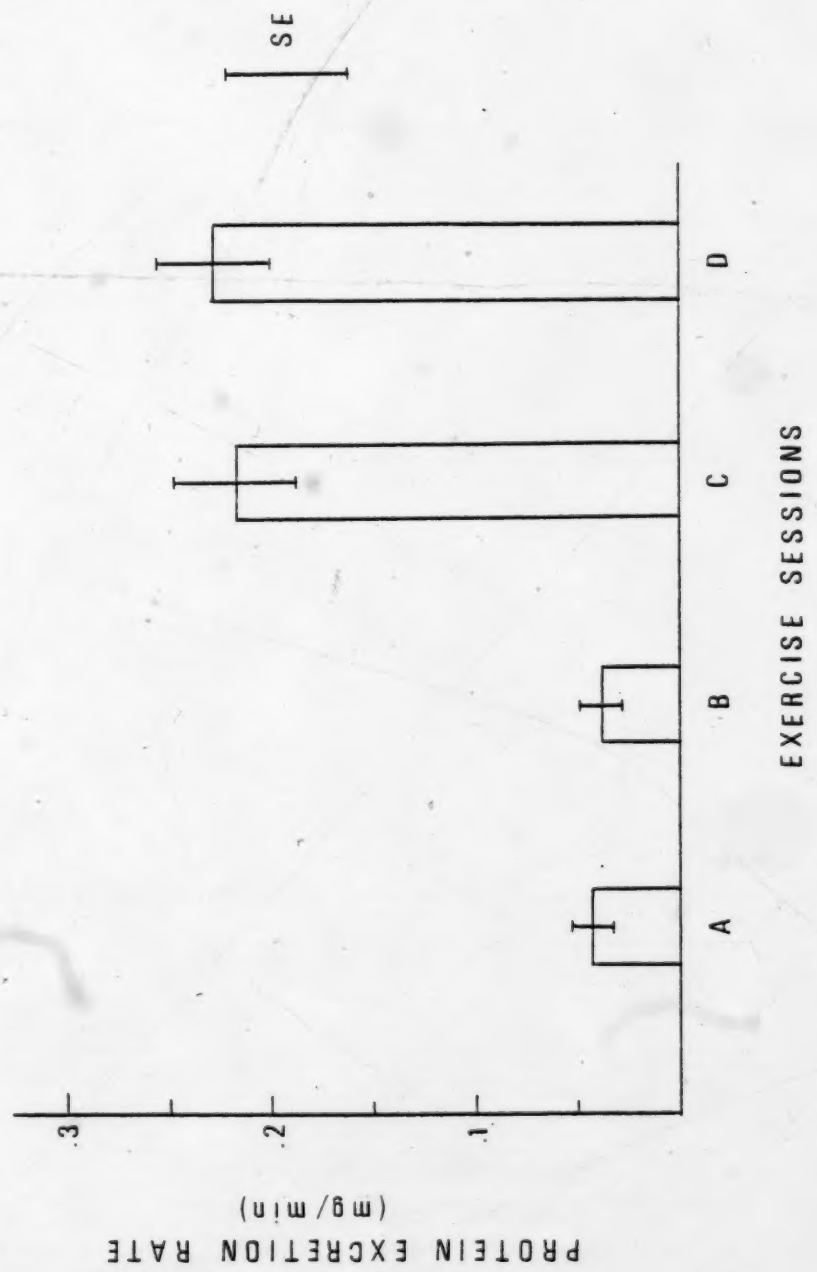
All subjects were within normotensive range and were free of renal abnormalities. The homogeneity of the sample is shown for several physical and functional characteristics, notably maximal power at maximal oxygen uptake, maximal oxygen uptake, height, weight, and body fat percentage.

The graphic representative (Figure 1) illustrates the protein excretion rates corresponding to the four exercise sessions. The mean values for the low intensity sessions A and B are .043 mg/minute and .038 mg/minute respectively, while the higher intensity sessions show values of .217 mg/minute for C and .228 mg/minute for D. The overall range of protein excretion rates for all four sessions was 0 to .385 mg/minute.

The post-hoc Scheffe analysis for the difference in means reveals no significant difference ($p > .05$) to exist between the protein excretion rates for the exercise sessions of similar intensity but varying duration, specifically between sessions A and B, and between sessions C and D. There is, however, significant difference between the various exercise sessions of disimilar intensities independent of the duration. These significant differences ($p < .01$) are between A and C, B and C, A and D, and B and D (see Appendix C-I).

The degree of variation for the protein excretion rates of the four treatments, as is indicated by percent coefficient of variation, is high for treatments A and B, 74.4% and 81.6% respectively and considerably lower for C (43.3%) and D (39.5%) (Appendix C-II).

Figure 1. The protein excretion rates are illustrated for urine samples collected 20 - 30 minutes following each of the four exercise sessions.


SE
.3
.2
.1
A
B
C
D
EXERCISE SESSIONS
PROTEIN EXCRETION RATE
(mg/min)

The urinary outputs, as shown in Appendix E, reveal similar mean values for both A and B exercise sessions, .913 and .814 ml/minute respectively. In addition, the mean values for C (.494 ml/minute) and D (.380 ml/minute) are also close. Comparing the value of the low intensity exercise sessions to the high intensity ones, reveals an approximately 2 to 1 relationship.

The oxygen uptake versus power relationship in a progressive-load test, as shown in Figure 2, indicates the classical plateauing effect in $\dot{M}VO_2$ attainment for the majority of subjects. According to an estimate of the oxygen uptake for a corresponding power increment, most subjects show an oxygen requirement of approximately 775 ml/minute for a power increment of 420 kp-m/minute. Maximal power loads corresponding to the $\dot{M}VO_2$ values is 2258 $\pm$ 157 kp-m/minute for all 10 cyclists.

The protein standards, as typically illustrated by the example in Figure 3, reveals that a high correlation exists between optical density and protein concentration for a range of concentrations from .25 mg to 2.0 mg in a constant volume of 4.2 ml ($r > .99$ for all protein standards over the course of analysis, a period of 4 months). For 30 repeated analyses of the forementioned range of protein concentrations, the percentage coefficients of variation, for any one protein concentration, are not greater than 2%.

The test for recovery of added protein shows the recovery percentages to range within 98.9 to 106.8% for the range of concentration (.063 to .5 mg/ml) studied (Appendix D). The test for recovery of added protein in dialysis analysis shows recovery percentages of 116 to 122%, whereby the total recovery was calculated, based on the volume of undialyzed sample. These high values show this method to overestimate the quantity of protein in dialysis.

Figure 2. Graph illustrates the relationship between oxygen uptake and power on a progressive-load maximal test.

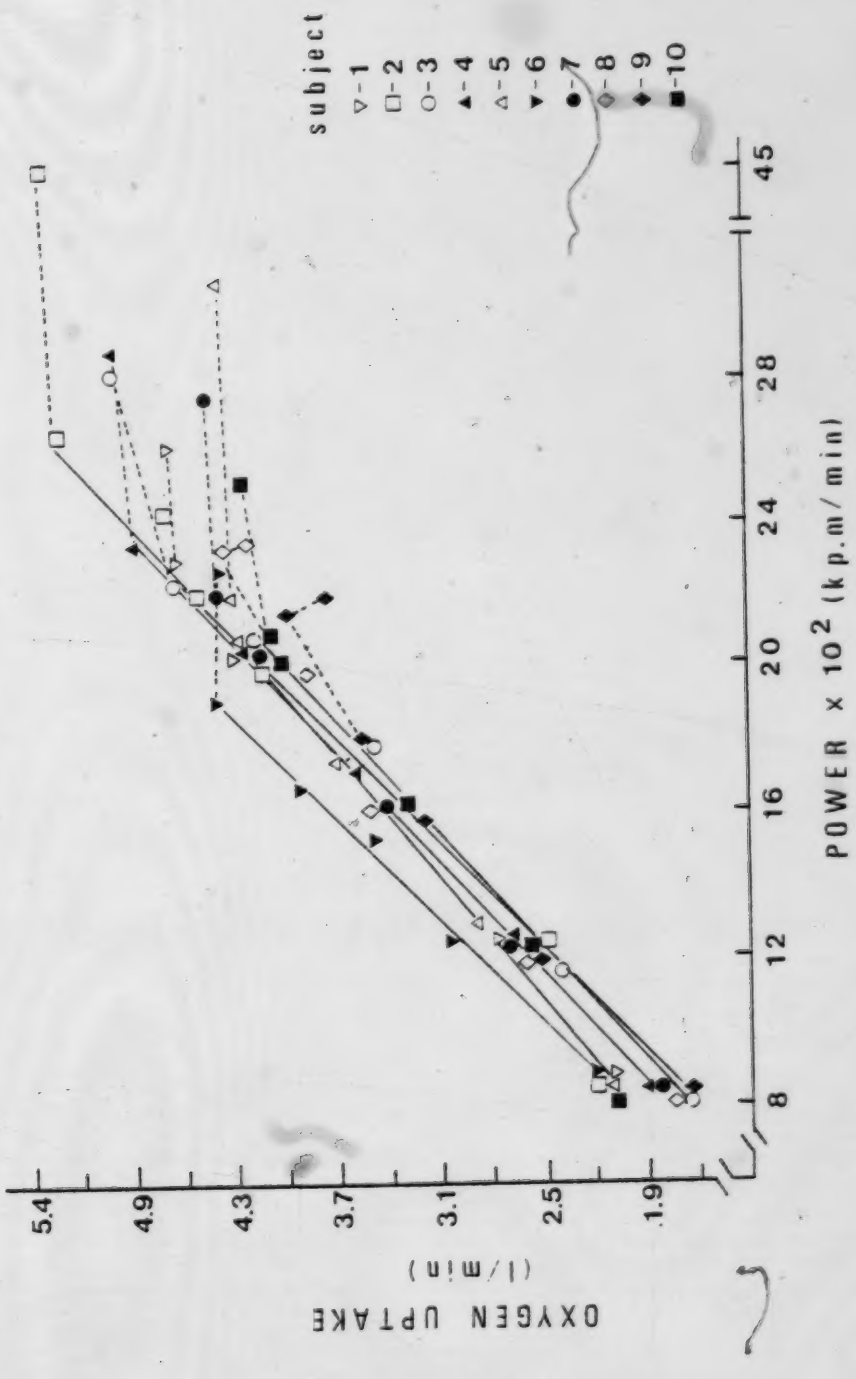
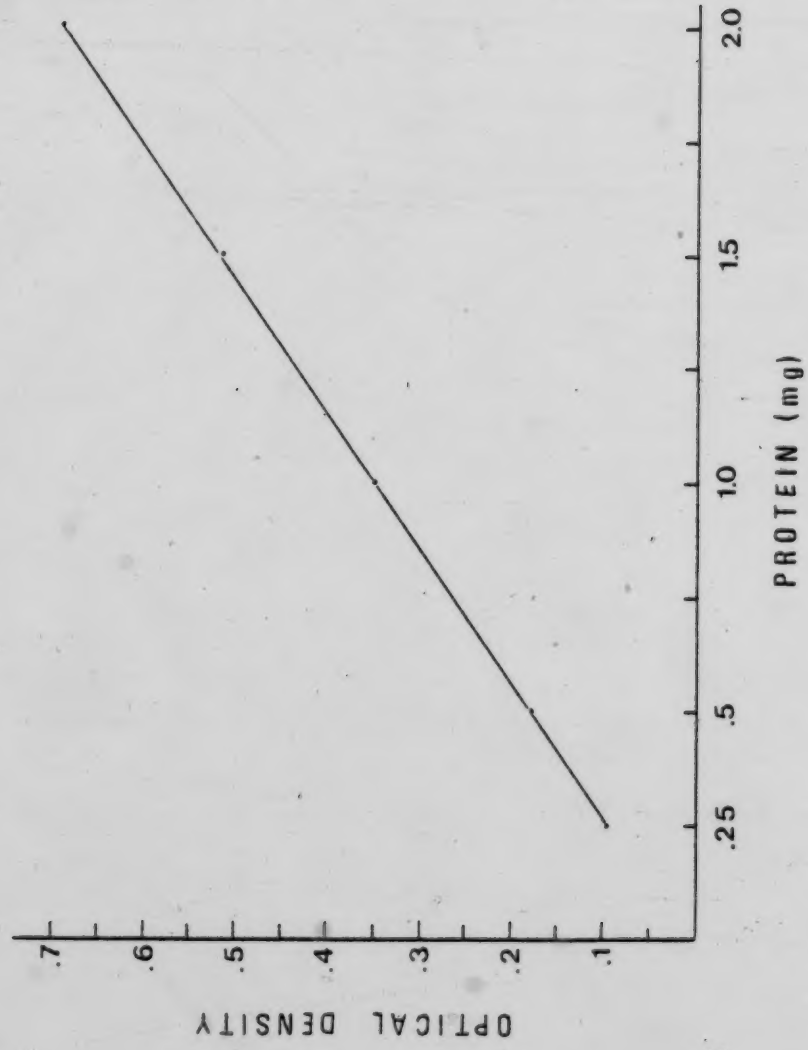

subject
1
2
3
4
5
6
7
8
9
10
OXYGEN UPTAKE
(l/min)
5.4
4.9
4.3
3.7
3.1
2.5
1.9
8
12
16
20
24
28
45
POWER x 10² (kp.m/min)

Figure 3. An example of a protein standard showing the relationship between optical density and protein quantity (all samples are in a constant volume of 4.2 ml). In this case, the linear regression equation for the prediction of protein quantity (y^1) from the known absorbance (x) is:

$$y^1 = -.0236 + 2.9340\ x$$


OPTICAL DENSITY
.7
.6
.5
.4
.3
.2
.1
PROTEIN (mg)
.25
.5
1.0
1.5
2.0

DISCUSSION

The effect of exercise intensity on the production of proteinuria is in contrast to the lack of effect of duration. However, when considering the total quantity of protein excreted over the period of exercise, then duration is an obvious factor.

The protein excretion rates for the low intensity exercise sessions A and B, approximate normal resting values (Appendix C-II). This suggests a threshold intensity may exist at which exercise proteinuria occurs. The finding by Perlman et al. (1970) indicating the total time of exercise for a maximal, progressive-load test to be correlated to the occurrence of exercise proteinuria, is suggestive of this hypothesis. It is unclear, though, whether the 50% $M\dot{V}O_2$ intensity as used in this study, would also elicit a low proteinuria quantity in an untrained population. The fact that a few individuals, such as subjects 7 and 9, did show somewhat elevated values for these two exercise sessions, further supports the suggestion that there exists threshold intensity for exercise proteinuria production. Moreover, the observed varied reaction to exercise by the cyclists can be accounted for by the existence of an inherent renal-constitutional difference or by the presence of a renal-adaptability characteristic.

The levels of exercise proteinuria seen with the higher intensity sessions C and D are comparable to the recovery samples taken within one hour from marathon runners, as cited by Poortmans and Jeanloz (1968). Providing both studies attained the "peak excretion rates", then it may be suggested that both groups were stressed at a similar relative

intensity. Other findings, such as those of Poortmans and Van Kerchove (1952) reported considerably higher proteinuria levels than those found in the present study. This may reflect the possibility that disproportionate increases in exercise proteinuria occur at high intensities, as is evidenced by Delforge et al. (1969). The observed differences in exercise proteinuria seen in various athletic groups, may also be explained by inherent renal-constitutional differences, a theory emphasized by Liljefors et al. (1969), as well as by adaptive differences possibly incurred through various types of testing regimes.

The degree of variability with treatments (Table 1) as is indicated by the percent coefficients of variation, is greatest for sessions A and B. As previously suggested, this may be due to the divided reactions of the group to exercise at this low intensity level. Results from the recovery test (Appendix D), suggests the procedure requiring microfiltration overestimates the protein excretion rates, and thus the ten dialized samples should not have contributed to the observed variation within the two treatments.

Generally, studies on proteinuria with exercise have reported wide dispersal of values with continuous exercise. The estimated percentage coefficient of variation within an exercise condition, typically range between 60 and 80%, as seen in various investigations (Liljefors et al., 1969; Poortmans and Jeanloz, 1968; Todorović et al., 1972). The lower values found in the present study for sessions C and D, may be indicative of the method by which work rate was established for the subjects. In this present study, it was found that the relative percentages of $M\dot{V}O_2$ i.e. target $\dot{V}O_2$ values, could be maintained within close limits, only slightly higher than the estimated 2% fluctuation found by Flint et al.

(1974). Therefore, any margin of variation in protein excretion rates, which would be accounted for by relative intensity differences amongst the subjects then possibly should be explained by factors such as training status or anaerobic threshold level. The accumulated work values (Appendix G) offer a general indication of the subjects' ability to maintain the target $\dot{V}O_2$ level without requiring adjustment of the work rate, and thus gives an insight into anaerobic threshold levels. The data of subject 1 and 3 suggests that they both have lower anaerobic thresholds, and show high protein excretion rates, while subjects 6 and 10 display possibly a greater anaerobic threshold level, and do show slightly lower protein excretion values. Such evidence may then be supportive of the importance of blood acidity in exercise proteinuria, as originally thought by Javitt (1952).

Literature indicates, as discussed by Bauman and Chinnard (1975), that within twenty days following a nephrectomy, there may be hypertrophy of 1.5 times for the remaining kidney, which subsequently resumes 80% of the body's total functional capacity in eight to twelve months. Of interest is the case of one of the subjects of the present study, who had undergone a nephrectomy five years prior, as a result of an accident. This subject, since that time, has not suffered any repercussions resulting from exercise, and has actively engaged in competition. His protein excretion rates of .044 mg/minute for session A and .100 mg/minute for session D, are somewhat supressed in comparison to the values for the other cyclists. Urinary outputs of .728 ml/minute and .349 ml/minute respectively, are comparable to those of the other cyclists. These results suggest the ability of the kidney to adapt by compensating, a situation which could have possibly been enhanced through training.

It was initially suspected that the low protein concentrations found for the 10 dialized samples in sessions A and B could have been due to a high diuretic effect as a result of low intensity exercise, as shown by Kachadorian and Johnson (1970). The results of the corresponding outputs (Appendix E) to the other values in sessions A and B do not substantiate this occurrence. It is difficult to compare the mean urinary outputs to normal 24 hour values due to a multitude of uncontrollable factors, such as level of prehydration, dietary factors, emotional state, and time of day. However, comparisons of the urinary outputs for the low intensity to that of the high intensity sessions reveals an approximate 2 to 1 ratio.

Possible explanations for exercise proteinuria seem all-encompassing, but have frequently favoured an "altered glomerular function". It is not clear, for instance, exactly what role amino acid reabsorption plays in exercise proteinuria. The linking of progressive events during exercise suggests need for further investigation. The known importance of amino acid catabolism in ammonia production (Owen and Robinson, 1963), ammonia's importance in acid-base balance in addition to its observed elevated excretion with exercise, suggests an increased reabsorption of amino acids with exercise. Furthermore, the observed preferential reabsorption of arginine following its infusion (Mogenson et al., 1975) results in increased protein excretion. Similarly, it may be speculated that amino acids are preferentially reabsorbed during exercise with subsequent increase in protein excretion as a result.

As has been shown by diverse investigations, exercise intensity occupies an important function in accounting for observed bodily changes (e.g. cardiovascular changes) during exercise, and this fact is further

substantiated by the present study. The findings of this study also link exercise intensity to the change in renal function seen with proteinuria. Future research, however, is required to establish the characteristics of exercise proteinuria through both the use of a larger range of relative intensities, as well as investigations throughout the recovery period following exercise. With such information, the implications of exercise proteinuria as a meter for exercise will be defined.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to determine the effect that intensity and duration of exercise have on the production of proteinuria. Secondary to this purpose, a case study was presented of a cyclist with a single kidney, so that insight may be gained on the adaptability of the renal system in handling protein under the stress of exercise.

The subjects, ten trained cyclists, were involved in three types of measurement situations; the orientation session, the test session, and the exercise session.

The orientation session involved the screening of the subjects for medical problems, the obtaining of anthropometric data, including body fat percentage and arterial pressures. The test session involved the testing of maximal oxygen uptake of the subjects. This was accomplished on a progressive load test at 70 rpm, allowing steady states to be attained on submaximal power loads, and then using the oxygen uptake requirements for increment of power as a criterion for further power increases during the near maximal stage of testing. A maximal power for $\dot{M}VO_2$ was determined and acted as the base for determining initial force settings during the exercise session.

Four exercise treatments (exercise sessions) were established -- 50% $\dot{M}VO_2$ for 30 minutes, 50% $\dot{M}VO_2$ for 60 minutes, 85% $\dot{M}VO_2$ for 17.5 minutes, and 85% $\dot{M}VO_2$ for 35 minutes, and these were randomly assigned for all ten subjects to complete. During these exercise sessions, subjects were monitored through collection of expired air, and through heart rate measurements, to ensure the target intensity was maintained. During all exercise sessions, subjects cycled at 70 rpm.

Samples of urine were taken as an initial marker following a five minute warm-up period at 40% $\dot{M}VO_2$. Urine samples were again taken 20 to 30 minutes following exercise, and were measured for protein by a precipitation technique outlined by Piscator (1962). Ten samples from exercise sessions A and B required concentrating, due to protein concentrations which were below the sensitivity of the planned method of analysis.

Protein excretion rates on the exercise sessions were analysed by a one-way analysis of variance and a post-hoc Scheffe test was performed on the significant means.

The results showed no significant difference in protein excretion rates between exercise sessions of the same intensity. There were significant differences ($p < .05$) for all combinations of exercise sessions of varying intensity on protein excretion rates.

In conclusion exercise intensity affects the level of exercise protein excretion rate while duration has little to no effect on this phenomenon. Also, there exists a level below which exercise proteinuria does not occur. Results of the individual with a single unilateral kidney show low exercise protein excretion rates in comparison to fellow cyclists. This indicates the adaptability of this individual's single kidney to handle the stress from exercise.

Further studies using greater numbers of exercise intensities may clarify certain characteristics of exercise proteinuria, which will aid in its possible application as an exercise stress indicator.

REFERENCES

Alyea, E.P., and Parish, H.H. Renal response to exercise - urinary findings. J.A.M.A., 1958, 167: 807-813.

Anderson, R.E. The significance of proteinuria in athletes. Sports Medicine, 1975, May/June: 133-135.

Aurell, M., and Vikgren, P. Plasma renin activity in supine muscular exercise. J. Appl. Physiol., 1971, 31: 839-841.

Barach, J.H. Physiological and pathological effects of severe exertion (the marathon race) on the circulatory and renal systems. Arch. Int. Med., 1910, 5: 382-405.

Barach, J.H. Evidences of nephritis and urinary acidosis. Amer. J. Med. Sci., 1920, 159: 398-402.

Bauman, Jr., J.W., and Chinard, F.P. Overview of renal function, in Renal Function. St. Louis: 1975, p. 9.

Berggård, I. Proteins, glycoproteins and mucopolysaccharides in normal human urine. I. Fractionation of non-dializable materials by ultrafiltration and by zone electrophoresis. Arkiv. Kemi., 1962, 18: 291-326.

Berggard, I., and Bearn, A.G. Excretion of haptoglobulin in normal urine. Nature, 1962, 195: 1311-1312.

Boesken, W.H., Kopf, K., and Scholl-Meyer, P. Differentiation of proteinuric diseases by discelectrophoretic molecular weight analysis of urinary proteins. Clin. Nephrol., 1973, 1: 311-318.

Bohrer, M.P., Deen, W.M. Robertson, C.R., and Brenner, B.M. Mechanism of angiotensin II-induced proteinuria in the rat. Am. J. Physiol., 1977, 233: F13-F21.

Boone, A.W., Haltwanger, E., and Chambers, R.L. Football hamaturia. J.A.M.A. 1955, 158: 1516-1517.

Boyarsky, S. Abstract of discussion in Alyea, E.P., and Boone, A.W. Urinary findings resulting from nontraumatic exercise. South. Med. J., 1957, 5: 905-910.

Boyce, W.H., Garvey, F.K., and Norfleet, Jr., C.M. Proteins and other biocolloids of urine in health and in calculous disease. I. Electrophoretic studies at pH 4.5 and 8.6 of those components soluble in molar sodium chloride. J. Clin. Invest., 1954, 33: 1287-1297.

Božović, L., Castenfors, J., and Piscator, M. Effect of prolonged, heavy exercise on urinary protein excretion and plasma renin activity. Acta. Physiol. Scand., 1967, 70: 143-146.

Cantone, A., and Cerretelli, P. Effect of training on proteinuria following muscular exercise. Int. Z. Angew. Physiol., 1960, 18: 324-329.

Castenfors, J. Renal function during exercise. Acta. Physiol. Scand., 1967, 70(Suppl. 293): 1-44.

Castenfors, J., Mossfeldt, F., and Piscator, M. Effect of prolonged heavy exercise on renal function and urinary protein excretion. Acta. Physiol. Scand., 1967, 70: 194-206.

Castenfors, J., and Piscator, M. Renal haemodynamics, urine flow and urinary protein excretion during exercise in supine position at different loads. Acta. Med. Scand., 1967, suppl. 472: 321-244.

Chambers, R., and Zweifach, B.W. Capillary endothelial cement in relation to permeability. J. Cell. Comp. Physiol., 1940, 15: 155-272.

Chang, R.L.S., Deen, W.M., Robertson, C.R., and Brenner, B.M. Permselectivity of the glomerular capillary wall: III. Restricted transport of polyanions. Kid. Int., 1975, 8: 212-218.

Chapman, C.B., Henschel, A., and Forsgren, A. Renal plasma flow during moderate exercise of several hours' duration in normal male subjects. Proc. Sco. Exp. Biol. Med., 1948, 69: 170-171.

Christensen, E.H., and Hogberg, P. Physiology of skiing. Int. Z. Angew. Physiol., 1950, 14: 292-303.

Collier, W. Functional albuminuria in athletes. Br. Med. J., 1907, 1: 4-6.

Coye, R.D., and Rosandich, R.R. Proteinuria during the 24-hour period following exercise. J. Appl. Physiol., 1960, 15: 592-594.

Delanne, R. Repercussion des activites musculaires sur le fonctionnement renal. Acta. Belg. Arte. Med. Pharm. Milit., 1957, 110: 363.

Delforge, E., Delforge, B., and Poortmans, J.R. Influence of increasing activity on the protein level in serum, urine and sweat in Poortmans, J.R. (Ed.), Biochemistry of Exercise, Medicine and Sport, Basel: S. Karger. 1969, pp. 353-360.

Deodhar, S.D., Cuppage, F.E., and Cableman, E. Studies on the mechanism of experimental proteinuria enduced by renin. J. Exp. Med., 1964, 120: 677-690.

Eisenbach, G.M., Van Liew, J.B., and Boylan, J.W. Effect of angiotensin on the filtration of protein in the rat kidney: A micropuncture study. Kid. Int., 1975, 8: 80-87.

Everall, P.H., and Wright, G.H. Low pressure ultra-filtration of protein-containing fluids. J. Med. Lab. Tech., 1958, 15: 209-213.

Fagard, R., Amery, A., Reybrouck, T., Lijnen, P., Moerman, E., Bogaert, M., and Schaepdryver, A. Effects of antiotensin antagonism on hemodynamics, renin, and catecholamines during exercise. J. Appl. Physiol., 1977: 43: 440-444.

Flint, M.M., Drinkwater, B.L., and Horvath, S.M. Effects of training on women's response to submaximal exercise. Med. Sci. Sports, 1974, 6: 89-94.

Free, A.H., and Free, H.M. Microscopic examination of urine sediment, in Urinalysis in Clinical Laboratory Practice. Cleveland: CRC Press. 1975, pp. 91-95.

Gardner, Jr., K.D. "Athletic pseudonephritis" - alteration of urine sediment by athletic competition. J.A.M.A., 1956, 161: 1613-1617.

Gardner, Jr., K.D. The effect of pH on the filtration, reabsorption and excretion of protein by the rat kidney. J. Clin. Invest., 1961, 40: 525-535.

Goa, J. A micro biuret method for protein determination: Determination of total protein in cerebrospinae fluid. Scand. J. Clin. Lab. Invest., 1953, 5: 218-222.

Göthlin, J., Sakuma, S., and Ishigaki, T. Effects of vasopressin in experimental nephroangrography. Acta. Radiol (Diag)., 1975, 16: 609-617.

Grant, G.H. The proteins of normal urine. II. From the urinary tract. J. Clin. Path., 1959, 12: 510-517.

Gray, I., and Beethan, Jr., W.P. Changes in plasma concentration of epinephrine and norepincphrine with muscular work. Pro. Soc. Expl. Biol. Med., 1957, 96: 636-638.

Grimby, G. Renal clearances during prolonged supine exercise at different loads. J. Appl. Physiol., 1965, 20: 1294-1298.

Hickson, R.C., Bomze, H.A., and Holloszy, J.O. Linear increase in aerobic power induced by a strenuous program of endurance exercise. J. Appl. Physiol., 1977, 42: 372-376.

Hori, S., Kurasiki, C., and Masumura, S. Kallikrein-induced proteinuria and angiotensin-induced proteinura. Mie Med. J., 1969, 19: 75-80.

Howley, E.T. The effect of different intensities of exercise on the excretion of epinephrine and norepinephrine. Med. Sci. Sports, 1976, 8: 219-222.

Issekutz, Jr., B., Birkhead, N.C., and Rodahl, K. Use of respiratory quotients in assessment of aerobic work capacity. J. Appl. Physiol., 1962, 17: 47-50.

Javitt, N.B., and Miller, Jr., A.T. Mechanism of exercise proteinuria. J. Appl. Physiol., 1952, 4: 834-839.

Kachadorian, W.A., and Johnson, R.E. Renal responses to various rates of exercise. J. Appl. Physiol., 1970, 28: 748-752.

Kachadorian, W.A., Johnson, R.E., Buffington, R.E., Lawler, L., Serbin, J.J., and Woodall, T. The regularity of "athletic pseudo-nephritis" after heavy exercise. Med. Sci. Sports, 1970, 2: 142-145.

Katch, V., Weltman, A., Sady, S., and Freedson, P. Validity of the relative percent concept for equating training intensity. Eur. J. Appl. Physiol., 1978, 39: 219-227.

King, S.E., and Baldwin, D.S. Production of renal ischemia and proteinuria in man by the adrenal medullary hormones. Am. J. Med., 1956, 20: 217-224.

Kleiman, A.H. Athlete's kidney. J. Urol., 1960, 83: 321-329.

Kotchen, T.A., Hartley, L.H., Rice, T.W., Mougey, E.H., Jones, L.G., and Mason, J.W. Renin, norepinephrine, and epinephrine responses to graded exercise. J. Appl. Physiol., 1971, 31: 178-184.

Kozlowski, S., Szczepanska, E., and Zielinski, A. The hypothalamo-hypophyseal antiduretic system in physical exercises. Arch. Int. Physiol. Bioch., 1967, 75: 218-228.

Liljefors, I., Piscator, M., and Risinger, C. Exercise proteinuria in monozygotic and dizygotic twins, in Poortmans, J.E. (ed.), Biochemistry of Exercise, Medicine and Sport. Basel: S. Karger. 1969, pp. 333-339.

McGarry, E., Sehon, A.H., and Rose, B. The isolation and electrophoretic characterization of the proteins in the urine of normal subjects. J. Clin. Invest., 1955, 34: 832-844.

McKay, E., and Slater, R.J. Studies on human proteinuria. II. Some characteristics of the gamma globulins excreted in normal exercise, postural and nephrotic proteinuria. J. Clin. Invest., 1962, 41: 1638-1652.

McKenzie, J.K., Patel, R., and McQueen, E.G. The excretion rate of Tamm-Horsfall urinary mucoprotein in normals and in patients with renal disease. Australas. Ann. Med., 1964, 13: 32-39.

McQueen, E.G. The nature of urinary casts. J. Clin. Path., 1962, 15: 367-373.

Mogenson, C.E., Vittinghus, E., and Sölling, K. Increased urinary excretion of albumin, light chains, and B_2-microglobulin after intravenous arginine administration in normal man. Lancet, 1975, 2: 581-583.

Mohos, S.C., and Skoza, L. Histochemical demonstration and localization of sialoproteins in the glomerulus. Exp. Mol. Pathol., 1970, 12: 316-323.

Murakami, N., Hori, S., and Masumura, S. Exercise proteinuria and proteinuria induced by kallikrein. Nature, 1968, 218: 481-483.

Nebdal, J., and Seliger, V. Electrophoretic analysis of exercise proteinuria. J. Appl. Physiol., 1958, 13: 244-246.

Owen, E.E., and Robinson, R.R. Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration of ammonium chloride. J. Clin. Invest., 1963, 42: 263-276.

Patel, R. Urinary casts in exercise. Australas. Ann. Med., 1964, 13: 170-173.

Perlman, L.V., Cunningham, D., Montoye, H., and Chiang, B. Exercise proteinuria. Med. Sci. Sports., 1970, 2: 20-23.

Pessina, A.C., Hulme, B., and Peart, W.S. Renin-induced proteinuria and the effects of adrenalectomy. II. Morphology in relation to function. Proc. R. Soc. Lond (Biol)., 1972, 180: 61-71.

Peterson, P.A., Evrin, P.-E., and Berggard, I. Differentiation of glomerular, subular and normal proteinuria: Determination of urinary excretion of B_2-microglobulin, albumin, and total protein. J. Clin. Invest., 1969, 48: 1189-1198.

Piscator, M. Proteinuria in chronic cadmium poisoning. Arch. Environ. Health, 1962, 5: 325-332.

Poortmans, J. La protéinurie physiologique du repose et a l'effort. Ann. Soc. Royale Sc. Méd. et Natur. de Bruxelles, 1964, 17: 89-188.

Poortmans, J.R. Urinary excretion of γM-related substance following muscular exercise. Nature, 1969, 221: 376-378.

Poortmans, J.R. Influence of physical exercise on proteins in biological fluids, in Poortmans, J.R. (Ed.), Biochemistry of Exercise, Medicine and Sport. Basel: S. Karger. 1969, pp. 312-327.

Poortmans, J.R. Serum protein determination during short exhaustive physical activity. J. Appl. Physiol., 1971, 30: 190-192.

Poortmans, J. Effect of exercise on the renal clearance of amylase and lysozyme in humans. Clin. Sci., 1972, 43: 115-120.

Poortmans, J.R. Effects of long lasting physical exercise and training on protein metabolism, in Howald, H., and Poortmans, J.R. (Eds.), Metabolic adaptation to prolonged physical exercise. Basel: Birkhäuser Verlag. 1975.

Poortmans, J., and Jeanloz, R.W. Quantitative immunological determination of 12 plasma proteins excreted in human urine collected before and after exercise. J. Clin. Invest., 1968, 47: 386-393.

Poortmans, J.R., and Jeanloz, R.W. Urinary excretion of immunoglobulins and their subunits in human subjects before and after exercise. Med. Sci. Sports., 1969, 1: 57-64.

Poortmans, J., and Segers, M. Haptoglobinuria following muscular exercise. Experientia, 1964, 20: 44.

Poortmans, J., and Van Kerchove, E. La proteinurie d'effort. Clin. Chim. Acta., 1962, 7: 229-242.

Poortmans, J.R., and Vancalck, B. Renal glomerular and subular impairment during strenuous exercise in young women. Eur. J. Clin. Invest., 1978, 8: 175-187.

Prejac, M., Maver, H. and Straus, B. Proteinuria of physical effort. Lijec. Vjesn., 1968, 90: 227-233.

Rigas, D.A., and Heller, C.G. The amount and nature of urinary proteins in normal human subjects. J. Clin. Invest., 1951, 30: 853-861.

Röcker, L., Kirsch, K., Mund, U., and Stoboy, H. The role of plasma proteins in the control of plasma volume during exercise and dehydration in long distance runners and cyclists, in Howald, H., and Poortmans, J.R. (Eds.), Biochemistry of Exercise. Basel: Birkhauser. 1975. pp. 238-244.

Rodewald, R., and Karnovsky, M.I. Porous substructure of the glomerular slit diaphragm in the rat and mouse. J. Cell Biol., 1974, 60: 423-433.

Saltin, B., and Stenberg, J. Circulatory response to prolonged severe exercise. J. Appl. Physiol., 1964, 19: 833-838.

Schultze, H.E., and Heremans, J.F. The urinary proteins. Chap. II. Nature and metabolism of extracellular proteins. in Molecular Biology of Human Proteins. New York: Elsevier Publ. Co. 1966, pp. 670-731.

Sellers, A.L., Smith, S., Marmorston, J., and Goodman, H.C. Studies on the mechanism of experimental proteinuria. J. Exp. Med., 1952, 96: 643-651.

Tamm, I., and Horsfall, Jr., F.L. Characterization and separation of an inhibitor of viral hemagglutination present in urine. Proc. Soc. Exp. Biol. Med., 1950, 74: 108-114.

Tamm, I., and Horsfall, Jr., F.L. A mucoprotein derived from human urine which reacts with influenza, mumps, and Newcastle disease viruses. J. Exp. Med., 1952, 95: 71-97.

Taylor, A. Some characteristics of exercise proteinuria. Clin. Sci., 1960, 19: 209-217.

Thorburn, G.D., Kapold, H.H., Herd, J.H., Hollenberg, M., O'Morghoe, C.C., and Barger, A.C. Intraenal distribution of nutrient blood flow determined with krypton^{85} in the unanesthetized dog. Circ. Res., 1963, 13: 290-307.

Tidström, B. Quantitative determination of protein in normal urine. Scand. J. Clin. Lab. Invest., 1963, 15: 167-172.

Todorovic, B., Nikolic, B., Brdaric, R., Nikolic, V., Stojadinovic, B., and Pavlovic-Kentera, V. Proteinuria following submaximal work of constant and variable intensity. Int. Z. Agnew. Physiol., 1972, 30: 151-160.

Tomasi, Jr., T.B., Tan, E.M., Solomon, A., and Prendergast, R.A. Characteristics of an immune system common to certain external secretions. J. Exp. Med., 1965, 121: 101-124.

White, H.L., and Rolf, D. Effects of exercise and of some other influences on the renal circulation in man. Am. J. Physiol., 1948, 152: 505-516.

Zaruba, K., and Fixa, B. Effect of severe physical exercise on the kidneys. Med. Exp., 1964, 11: 233-238.

APPENDICES

Appendix A-I	Physical Characteristics of Subjects
Appendix A-II	Functional Characteristics of Subjects
Appendix B	Health Questionnaire Form
Appendix C-I	Scheffe Comparison of Mean Differences for Protein Excretion Rates During Four Exercise Sessions
Appendix C-II	Protein Excretion Rates Following Exercise
Appendix C-III	Summary of One-Way ANOVA on Protein Excretion Rates During Exercise Sessions
Appendix D-I	Tests for Recovery of Added Protein Over the Course of Analysis
Appendix D-II	Test for Recovery of Added Protein in Dialysis Analysis
Appendix E-I	Raw Data - Exercise Session A
Appendix E-II	Raw Data - Exercise Session B
Appendix E-III	Raw Data - Exercise Session C
Appendix E-IV	Raw Data - Exercise Session D
Appendix F	Sample Data from Test Session
Appendix G	Total Work Accumulated for Exercise Sessions
Appendix H-I	Physical and Functional Characteristics for Case Study
Appendix H-II	Raw Data for Case Study
Appendix I	Details of Chemical Constituents in Protein Analysis

APPENDIX A

Appendix A-I. Physical Characteristics of Subjects

Appendix A-II. Functional Characteristics of Subjects

APPENDIX A-I

Physical Characteristics of Subjects

Subject	Age (yrs)	Height (cm)	Weight (kg)	Body Fat (%)	Arterial Pressure (mm Hg)
1	23	177.8	74.5	5.1	132/74
2	25	184.9	80.4	13.3	124/75
3	31	177.8	79.0	6.6	128/80
4	25	180.3	79.5	3.9	128/82
5	29	168.9	65.7	8.2	115/70
6	32	175.3	69.1	9.3	124/68
7	22	182.4	73.9	7.8	134/80
8	19	174.0	69.9	9.0	128/74
9	22	186.2	77.1	5.1	135/70
10	27	174.8	77.5	10.9	122/68
MEAN	25.5	178.2	74.7	7.9	127/74
SD	±4.2	±5.3	±5.0	±2.9	±6/±5.2

APPENDIX A-II

Functional Characteristics of Subjects

Subject	$\dot{M}VO_2$ (ml/min)	$\dot{M}VO_2$ ($ml\ kg^{-1}min^{-1}$)	MPO* kp·m/min	Max Heart Rate (bpm)
1	4701	63.1	2250	180
2	5400	67.2	2730	180
3	5118	64.8	2580	188
4	4979	62.6	2500	196
5	4394	66.9	2170	-
6	4411	63.8	1950	185
7	4455	60.3	2200	198
8	4343	62.1	2150	200
9	3985	51.7	1950	-
10	4271	55.1	2100	-
MEAN	4606	61.8	2258	
SD	±265	±1.8	±157	

- Represents Missing Values

* Maximal Power at $\dot{M}VO_2$

APPENDIX B

Health Questionnaire Form

Health Questionnaire

Name: ..... Birthdate: .....

MEDICAL HISTORY

Do you or have you ever suffered from:	(Please Specify)	
Heart Trouble	yes	no
High Blood Pressure	yes	no
Lung Disease	yes	no
Kidney Disorders	yes	no
Urinary Infections	yes	no
Diabetis Mellitus	yes	no
Diabetis Insipidus	yes	no
Inborm Errors of Metabolism	yes	no
Ateriosclerosis/Atherosclerosis	yes	no
Loss or Absence of a Kidney	yes	no

Others .....

.....

Occupation: .....

Do you come in contact with chemicals on your job? (e.g. mercurials, acetazolamide, chlorothiazide, narcotics) .....

.....

Are you presently on medication? (Please Specify) .....

.....

Do you smoke? ..... packs/week

Drink coffee or tea? ..... cups/day

Drink Alcohol? ..... drinks/week

During the participation of this project, will you be administered anesthetics of any kind? (e.g. Dentist) .....

Do you ever experience problems with urination? .....

Have you ever been told that you had a urine sample which proved not normal? .....

Appendix B (Continued)

Have you ever noticed peculiarities with your urine, such as in colour, odour, or appearance of materials? .....

Please specify on your training as to quantity and quality .....

.....

.....

.....

APPENDIX C

Appendix C-I. Scheffe Comparison of Mean Differences for Protein Excretion Rates During Four Exercise Sessions

Appendix C-II. Protein Excretion Rates Following Exercise

Appendix C-III. Summary of One-Way ANOVA on Protein Excretion Rates During Exercise Sessions

APPENDIX C-I

Scheffe Comparison of Mean Differences for Protein Excretion Rates During Four Exercise Sessions

		A	B	C	D
	MEANS	.043	.038	.218	.228
A	.043	0	.005	.175*	.185*
B	.038		0	.180*	.190*
C	.218			0	.010
D	.228				0

* p .01

APPENDIX C-II

Protein Excretion Rates
Following Exercise

Subject	Exercise Session A	B	C	D
1	.023*	.019*	.210	.378
2	0*	0*	.385	.205
3	.024*	.022*	.279	.368
4	.020*	.021*	.343	.238
5	.028*	.015*	.191	.153
6	.103	.102	.064	.148
7	.069	.053	.170	.261
8	.030	.057	.210	.166
9	.078	.065	.170	.245
10	.055	.026	.149	.115
MEAN	.043	.038	.217	.228
SD	±.032	±.031	±.094	±.090
% C.V.	74.4%	81.6%	43.3%	39.5%

* Samples Which Were Dialized Prior to Analysis.

% C.V. (% of coefficient variation) = $\frac{SD}{MEAN}$

APPENDIX C-III

Summary of One-Way ANOVA on Protein Excretion Rates During Exercise Sessions

Source of Variation	SS	DF	MS	F
Between People	.03035629	9	.0033729207	
Within People	.47252536	30	.015750844	21.1308*
Treatments	.331383	3	.11046112	
Residual	.14114201	27	.0052274801	
Total	.50288165	39		

Probability of F = .00000 DF = (k - 1)/(N - 1)(k - 1)

Conservative Probability = .00130 DF = 1/(N - 1)

Critical $3F_{.99}(3,27) = 3(4.61) = 13.83$

* $F \frac{\text{treatment}}{\text{residual}}$ is significant at $p < .01$

APPENDIX D

Appendix D-I. Tests for Recovery of Added Protein Over the Course of Analysis

Appendix D-II. Test for Recovery of Added Protein in Dialysis Analysis

Appendix Figure 1. Sample Protein Standard

APPENDIX D-I

Tests for Recovery of Added Protein
Over the Course of Analysis

Beginning of Study

Known[a] Quantity Protein (mg)	Recovery[b] Protein (mg)	% Recovered
.25	.249	99.6%
.5	.507	101.4%
1.0	1.006	100.6%
1.5	1.484	98.9%
2.0	2.013	100.7%

End of Study

Known[a] Quantity Protein (mg)	Recovery[c] Protein (mg)	% Recovered
.25	.267	106.8%
.5	-	-
1.0	1.046	104.6%
1.5	1.493	99.5%
2.0	1.982	99.1%

a all protein quantities are in a volume of 4 ml.

b calculated with protein standard $y^1 = -.0236 + 2.934x$

c calculated with protein standard $y^1 = -.0111 + 3.0563x$

APPENDIX D-II

Test for Recovery of Added Protein
in Dialysis Analysis

Initial Protein Concentration 1 mg in 100 ml

Samples	Undialized Sample (mls)	Dialized Sample (mls)	Recovery[ab] Protein (mg)	% Recovery
1	6.45	85	1.167	116.7%
2	5.73	87	1.163	116.3%
3	6.75	86	1.215	122%

a calculated with protein standard $y^1 = -.0111 + 3.0563x$

b determinations based on volume of undialized sample

APPENDIX E

Appendix E-I. Raw Data - Exercise Session A

Appendix E-II. Raw Data - Exercise Session B

Appendix E-III. Raw Data - Exercise Session C

Appendix E-IV. Raw Data - Exercise Session D

APPENDIX E-I

Raw Data

Exercise Session A

Subject	Sample Vol. (ml)	Sample Time (min)	Total Protein (mg)	Protein Excretion (mg/ml)	Protein Excretion (mg/min)	Urinary Output (ml/min)
1	50	50.6	1.153	.023	.023	.988
2	44	60.3	-	-	-	.730
3	27.5	51.5	1.223	.044	.024	.534
4	34.0	52.6	1.030	.030	.020	.646
5	85.0	51.7	1.439	.017	.028	1.644
6	72.0	51.2	5.274	.073	.103	1.406
7	62.5	52.0	3.574	.057	.069	1.202
8	9.0	58.3	1.746	.194	.030	.154
9	51.8	51.7	4.027	.078	.078	1.002
10	43.2	52.7	2.905	.067	.055	.820
MEAN	47.9	53.3				.913
SD	±22.0	±3.3				±.436

APPENDIX E-II

Raw Data

Exercise Session B

Subject	Sample Vol. (ml)	Sample Time (min)	Total Protein (mg)	Protein Excretion (mg/ml)	Protein Excretion (mg/min)	Urinary Output (ml/min)
1	41	81.0	1.514	.038	.019	.506
2	28.5	87.3	-	-	-	.327
3	68.7	81.6	1.779	.026	.022	.842
4	72.9	81.0	1.667	.023	.021	.900
5	64.5	81.8	1.227	.019	.015	.789
6	125	82.0	8.403	.067	.102	1.524
7	65	81.0	4.305	.066	.053	.802
8	84	80.2	4.569	.054	.057	1.047
9	63	80.8	5.236	.083	.065	.780
10	51.2	81.7	2.099	.041	.026	.627
MEAN	66.4	81.8				.814
SD	±26.1	±2.0				±.322

- sample below the sensitivity of the method used for measurement

APPENDIX E-III

Raw Data

Exercise Session C

Subject	Sample Vol. (ml)	Sample Time (min)	Total Protein (mg)	Protein Excretion (mg/ml)	Protein Excretion (mg/min)	Urinary Output (ml/min)
1	13.8	40.6	8.531	.618	.210	.340
2	31.5	50.4	19.424	.912	.385	.422
3	25.6	39.0	10.895	.425	.279	.656
4	18.4	38.9	13.356	.725	.343	.473
5	29.5	37.4	3.804	.242	.191	.789
6	23.0	38.4	2.447	.107	.064	.599
7	11.1	37.3	6.334	.570	.170	.298
8	9.0	42.1	8.860	.981	.210	.214
9	24.4	38.4	6.515	.268	.170	.635
10	19.8	38.2	5.679	.288	.149	.518
MEAN	20.6	40.1				.494
SD	±7.6	±3.9				±.180

APPENDIX E-IV

Raw Data

Exercise Session D

Subject	Sample Vol. (ml)	Sample Time (min)	Total Protein (mg)	Protein Excretion (mg/ml)	Protein Excretion (mg/min)	Urinary Output (ml/min)
1	25.3	60.0	22.674	.896	.378	.422
2	26.3	64.7	13.269	.505	.205	.406
3	22.9	57.7	21.237	.927	.368	.397
4	43.6	56.4	13.431	.308	.238	.773
5	24.0	56.6	8.635	.361	.153	.424
6	20.5	56.5	8.385	.408	.148	.363
7	17.2	57.1	14.898	.867	.261	.301
8	10.4	65.3	10.819	1.044	.166	.159
9	16.6	56.0	13.698	.828	.245	.296
10	15.0	57.6	6.627	.442	.115	.260
MEAN	22.2	58.8				.380
SD	±9.1	±3.5				±.162

APPENDIX F

Sample Data From Test Session

APPENDIX F

Sample Data From Test Session

Subject #7

Power (kp.m/min)	$\dot{V}O_2$ (l/min)
840	1828
1251	2699
1632	3428
2010	4157
2220	4357
2740	4455*
2829	4362

* $M\dot{V}O_2$ value

APPENDIX G

Appendix G. Total Work Accumulated

APPENDIX G

Total Work (kp.m) Accumulated for Exercise Sessions

During Exercise

Exercise Sessions

Subject	A	B	C	D
1	33400	68000	29900	56500
2	41400	81100	37600	68400
3	36100	74500	32200	65000
4	39400	71000	33600	67000
5	29900	65500	28700	57700
6	27200	53000	27800	55100
7	34400	62800	30900	64600
8	31500	64400	29000	57200
9	28300	58400	27000	50600
10	29200	58500	29200	54200
MEAN	34100	65700	30600	59600
SD	±4900	±8300	±3200	±6100

APPENDIX H

Appendix H-I. Physical and Functional Characteristics

Appendix H-II. Raw Data for Case Study

APPENDIX H-I

Physical and Functional Characteristics for Case Study

Subject with Previous Nephrectomy	
Age (yrs)	24
Height (cm)	173.4
Weight (kg)	59.3
Body Fat (%)	7.0%
Arterial Pressure (mm Hg)	110/74
$\dot{M}VO_2$ (l/min)	3995
$\dot{M}VO_2$ (ml $kg^{-1}min^{-1}$)	67.5
MPO (kp -m/min)	1950

APPENDIX H-II

Raw Data - Case Study

Exercise Session	Sample Vol. (ml)	Sample Time (min)	Total Protein (mg)	Protein Excretion (mg/ml)	Protein Excretion (mg/min)	Urinary Output (ml/min)
A	37.5	51.5	2.242	.059	.044	.728
D	19.9	57.0	5.692	.286	.100	.349

APPENDIX I

Appendix I. Details on Chemical Constituents in Protein Analysis

APPENDIX I

Details On Chemical Constituents In Protein Analysis

Tsuchiya's Reagent:

Phosphotungstic Acid:	15 gm
Concentrated Hydrochloric Acid:	60 gm
95% Ethyl Alcohol:	770 ml
Distilled Water:	60 ml

Benedict's Reagent

A.

Sodium Citrate:	173 gm
Sodium Carbonate:	100 gm
Distilled Water:	500 ml

B.

Copper Sulfate:	17.3 gm
Distilled Water:	100 ml

- Heat solution "A" to dissolve contents then filter
- Combine "A" and "B"
- Add distilled water up to 1000 ml
- Store in dark bottles